```
Dialog leel 99.12.23D

Lat logoff:  01feb00 15:23:57
Logon file001 02feb00 16:04:28
*** ANNOUNCEMENT ***
NEW
***Kompa Mexico (File 586)
***Market Gide Compan Financial (File 100)
***Frot & Sllian Market Engineering  (File 767)
                          ***
UPDATING RESUMED
***Federal New Serice (File 660)
                          ***
RELOADED
***CLAIMS(r)/U.S. Patent (File 340,341,942)
***Gale Grop PROMT (File 16, 160)
***Gale Grop F&S Index (File 18)
***RAPRA (File 323)
***Gale Grop New Prodct Annoncement (File 621)

REMOVED
***The Colmb Dipatch (File 495)
***A-V Online (File 46)
***BNA Dail (File 655)
□dialog
     >>> Enter BEGIN HOMEBASE for Dialog Announcements <<<
     >>>    of new databases, price changes, etc.      <<<
*** ANNOUNCEMENT ***
Dialog Alerts for January 1, 2000 will be run on January 2
to minimize delivery delays due to scheduled network and IT
systems testing being performed by our customers on Jan 1.
                    ****
For news about price changes for Jan 1, 2000, enter
HELP NEWRATES.
                    ****

File   1:ERIC  1966-1999/Dec
       (c) format only 2000 The Dialog Corporation
*File   1: File has been reloaded. See HELP NEWS 1.
Limits of /ED and /EJ currently not working.

      Set  Items  Description
      ---  -----  -----------
? b 410

>>>'IALOG' not recognized as set or accession number
? set hi ;set hi

       02feb00 16:04:34 User208760 Session D1433.1
            $0.37    0.105 DialUnits File1
     $0.37  Estimated cost File1
     $0.05  TYMNET
     $0.42  Estimated cost this search
     $0.42  Estimated total session cost   0.105 DialUnits

File 410:Chronolog(R)  1981-2000 Jan/Feb
       (c) 2000 The Dialog Corporation plc

      Set  Items  Description
```

```
      ---  -----  -----------
?
HILIGHT set on as ''
HILIGHT set on as ''
? begin 652,653,654

       02feb00 16:04:50 User208760 Session D1433.2
            $0.00    0.050 DialUnits File410
     $0.00  Estimated cost File410
     $0.01  TYMNET
     $0.01  Estimated cost this search
     $0.43  Estimated total session cost   0.155 DialUnits

SYSTEM:OS  - DIALOG OneSearch
  File 652:US Patents Fulltext  1971-1979
        (c) format only 2000 The Dialog Corp.
*File 652: Reassignment data current through 12/06/1999 recordings.
Reexamination, extension, expiration, reinstatement updated weekly.
  File 653:US Pat.Fulltext  1980-1989
        (c) format only 2000 Knight-Ridder Info
*File 653: Reassignment data current through 12/069/1999 recordings.
Reexamination, extension, expiration, reinstatement updated weekly.
  File 654:US Pat.Full.  1990-2000/Jan 25
        (c) format only 2000 The Dialog Corp.
*File 654: Reassignment data current through 12/06/1999 recordings.
Reexamination, extension, expiration, reinstatement updated weekly.

      Set  Items  Description
      ---  -----  -----------
? s (cd40L or cd40(w)ligand or gp39)(40n)(link? or conjugat? or covalent or
fusion)(40n)(antigen? or immunogen? or vaccin?)

Processing
             50  CD40L
            287  CD40
          25719  LIGAND
            115  CD40(W)LIGAND
             48  GP39
         416347  LINK?
          58921  CONJUGAT?
          22699  COVALENT
          51323  FUSION
          33407  ANTIGEN?
          12214  IMMUNOGEN?
          12473  VACCIN?
      S1     34  (CD40L OR CD40(W)LIGAND OR GP39)(40N)(LINK? OR CONJUGAT?
                 OR COVALENT OR FUSION)(40N)(ANTIGEN? OR IMMUNOGEN? OR
                 VACCIN?)
? t s1/3/all


 1/3/1     (Item 1 from file: 653)
DIALOG(R)File 653:US Pat.Fulltext
(c) format only 2000 Knight-Ridder Info. All rts. reserv.

            01610323
Utility
PROTEINACEOUS      ANTIGENS      WITH      CONFORMATION-INDEPENDENT      AND
CONFORMATION-DEPENDENT DETERMINANTS

PATENT NO.:  4,683,136
ISSUED:      July 28, 1987 (19870728)
INVENTOR(s): Milich, David, Mira Mesa, CA (California), US (United States
             of America)
             Chisari, Frank, Del Mar, CA (California), US (United States of
             America)
```

ASSIGNEE(s): Scripps Clinic and Research Foundation, (A U.S. Company or Corporation), La Jolla, CA (California), US (United States of America)
[Assignee Code(s): 3325]
EXTRA INFO: Assignment transaction [Reassigned], recorded November 15, 1991 (19911115)
Assignment transaction [Reassigned], recorded November 18, 1991 (19911118)
Expired, effective August 2, 1995 (19950802), recorded in O.G. of October 10, 1995 (19951010)
Expired, effective July 28, 1999 (19990728), recorded in O.G. of October 5, 1999 (19991005)
APPL. NO.: 6-708,746
FILED: March 06, 1985 (19850306)
FULL TEXT: 1513 lines

1/3/2 (Item 1 from file: 654)
DIALOG(R)File 654:US Pat.Full.

03072903
Utility
ACTIVATED DENDRITIC CELLS AND METHODS FOR THEIR ACTIVATION

PATENT NO.: 6,017,527
ISSUED: January 25, 2000 (20000125)
INVENTOR(s): Maraskovsky, Eugene, Seattle, WA (Washington), US (United States of America)
Mc Kenna, Hilary J., Seattle, WA (Washington), US (United States of America)
ASSIGNEE(s): Immunex Corporation, (A U.S. Company or Corporation), Seattle, WA (Washington), US (United States of America)
[Assignee Code(s): 9809]
APPL. NO.: 8-763,995
FILED: December 12, 1996 (19961212)

This is a continuation of application Ser. No. 08-677,762, filed Jul. 10, 1996,now abandoned.

FULL TEXT: 1078 lines

1/3/3 (Item 2 from file: 654)
DIALOG(R)File 654:US Pat.Full.

03068024
Utility
DNA ENCODING TUMOR NECROSIS RELATED RECEPTOR TR7

PATENT NO.: 6,013,476
ISSUED: January 11, 2000 (20000111)
INVENTOR(s): Deen, Keith Charles, Glenmoore, PA (Pennsylvania), US (United States of America)
Hurle, Mark R., Norristown, PA (Pennsylvania), US (United States of America)
Young, Peter, Lawrenceville, NJ (New Jersey), US (United States of America)
Tan, K. B., Philadelphia, PA (Pennsylvania), US (United States of America)
ASSIGNEE(s): SmithKline Beecham Corporation, (A U.S. Company or Corporation), Philadelphia, PA (Pennsylvania), US (United States of America)
[Assignee Code(s): 23499]
APPL. NO.: 8-959,382

FILED: October 28, 1997 (19971028)
This application claims the benefit of U.S. Provisional Application No. 60-041,796, filed Apr. 2, 1997.

FULL TEXT: 1249 lines

1/3/4 (Item 3 from file: 654)
DIALOG(R)File 654:US Pat.Full.

03058244
Utility
ANTIGEN-CARRYING MICROPARTICLES AND THEIR USE IN THE INDUCTION OF HUMORAL OR CELLULAR RESPONSES

PATENT NO.: 6,004,763
ISSUED: December 21, 1999 (19991221)
INVENTOR(s): Gengoux, Christine, Argenteuil, FR (France)
Leclerc, Claude, Paris, FR (France)
ASSIGNEE(s): Institut Pasteur, (A Non-U.S. Company or Corporation), FR (France)
[Assignee Code(s): 42312]
APPL. NO.: 9-76,646
FILED: May 12, 1998 (19980512)
PRIORITY: 92-10879, FR (France), September 11, 1992 (19920911)

This application is a continuation-in-part of Ser. No. 08-397,286, filed Apr. 28, 1995 now U.S. Pat. No. 5,871,747.

FULL TEXT: 1210 lines

1/3/5 (Item 4 from file: 654)
DIALOG(R)File 654:US Pat.Full.

03042652
Utility
HETEROCYCLO-SUBSTITUTED IMIDAZOPYRAZINE PROTEIN TYROSINE KINASE INHIBITORS

PATENT NO.: 5,990,109
ISSUED: November 23, 1999 (19991123)
INVENTOR(s): Chen, Ping, Lawrenceville, NJ (New Jersey), US (United States of America)
Norris, Derek J., Trenton, NJ (New Jersey), US (United States of America)
Barrish, Joel C., Holland, PA (Pennsylvania), US (United States of America)
Iwanowicz, Edwin J., Cranbury, NJ (New Jersey), US (United States of America)
ASSIGNEE(s): Bristol-Myers Squibb Co , (A U.S. Company or Corporation), New York, NY (New York), US (United States of America)
[Assignee Code(s): 22921]
APPL. NO.: 9-262,525
FILED: March 04, 1999 (19990304)

This application claims priority from provisional U.S. application Ser. No. 60-076,789, filed Mar. 4, 1998, which is incorporated herein by reference in its entirety.

FULL TEXT: 2920 lines

1/3/6 (Item 5 from file: 654)
DIALOG(R)File 654:US Pat.Full.

03033571

Utility
DNA ENCODING CD40 LIGAND, A CYTOKINE THAT BINDS CD40

PATENT NO.: 5,981,724
ISSUED: November 09, 1999 (19991109)
INVENTOR(s): Armitage, Richard J., Bainbridge Island, WA (Washington), US (United States of America)
Fanslow, William C., Federal Way, WA (Washington), US (United States of America)
Spriggs, Melanie K., Seattle, WA (Washington), US (United States of America)
Srinivasan, Subhashini, Kirkland, WA (Washington), US (United States of America)
Gibson, Marylou G., Carlsbad, CA (California), US (United States of America)
Morris, Arvia E., Seattle, WA (Washington), US (United States of America)
McGrew, Jeffrey T., Seattle, WA (Washington), US (United States of America)
ASSIGNEE(s): Immunex Corporation, (A U.S. Company or Corporation), Seattle, WA (Washington), US (United States of America)
[Assignee Code(s): 9809]
APPL. NO.: 8-477,733
FILED: June 07, 1995 (19950607)

CROSS REFERENCE TO RELATED APPLICATION

This application is a continuation-in-part application of U.S. patent application Ser. No. 08-249,189, filed May 24, 1994, which is a continuation-in-part of U.S. patent application Ser. No. 07-969,703, filed Oct. 23, 1992, now abandoned, which is a continuation-in-part of U.S. patent application Ser. No. 07-805,723, filed on Dec. 5, 1991, now abandoned, which is a continuation-in-part of U.S. patent application Ser. No. 07-783,707, filed on Oct. 25, 1991, now abandoned.

FULL TEXT: 3829 lines

1/3/7 (Item 6 from file: 654)
DIALOG(R)File 654:US Pat.Full.

03028136

Utility
IMMUNOSTIMULATORY COMPOSITIONS

PATENT NO.: 5,976,546
ISSUED: November 02, 1999 (19991102)
INVENTOR(s): Laus, Reiner, San Carlos, CA (California), US (United States of America)
Ruegg, Curtis Landon, San Carlos, CA (California), US (United States of America)
Wu, Hongyu, Palo Alto, CA (California), US (United States of America)
ASSIGNEE(s): Dendreon Corporation, (A U.S. Company or Corporation), Seattle , WA (Washington), US (United States of America)
[Assignee Code(s): 46465]
APPL. NO.: 9-146,283
FILED: September 03, 1998 (19980903)

The application is a divisional of U.S. patent application Ser. No. 08-579,823 filed Dec. 28, 1995, now pending.
FULL TEXT: 1293 lines

1/3/8 (Item 7 from file: 654)
DIALOG(R)File 654:US Pat.Full.

03022493

Utility
ABSORPTION-ENHANCED DIFFERENTIAL EXTRACTION DEVICE

PATENT NO.: 5,971,158
ISSUED: October 26, 1999 (19991026)
INVENTOR(s): Yager, Paul, Seattle, WA (Washington), US (United States of America)
Brody, James P., Seattle, WA (Washington), US (United States of America)
ASSIGNEE(s): University of Washington, (A U.S. Company or Corporation), Seattle, WA (Washington), US (United States of America)
[Assignee Code(s): 2937]
APPL. NO.: 8-876,038
FILED: June 13, 1997 (19970613)

CROSS-REFERENCE TO RELATED APPLICATIONS

This is a utility patent application taking priority from provisional patent application Ser. No. 60-019,904 filed Jun. 14, 1996, which is incorporated herein in its entirety by reference.

This invention was made with government support under Army research contract DAMD17-94-4460 awarded by the U.S. Army. The government has certain rights in the invention.

FULL TEXT: 1443 lines

1/3/9 (Item 8 from file: 654)
DIALOG(R)File 654:US Pat.Full.

03012748

Utility
RECOMBINANT SOLUBLE CD40 LIGAND POLYPEPTIDE AND PHARMACEUTICAL COMPOSITION CONTAINING THE SAME

PATENT NO.: 5,962,406
ISSUED: October 05, 1999 (19991005)
INVENTOR(s): Armitage, Richard J., Bainbridge Island, WA (Washington), US (United States of America)
Fanslow, William C., Federal Way, WA (Washington), US (United States of America)
Spriggs, Melanie K., Seattle, WA (Washington), US (United States of America)
Srinivasan, Subhashini, Kirkland, WA (Washington), US (United States of America)
Gibson, Marylou G., Carlsbad, CA (California), US (United States of America)
Morris, Arvia E., Seattle, WA (Washington), US (United States of America)
McGrew, Jeffrey T., Seattle, WA (Washington), US (United States of America)
ASSIGNEE(s): Immunex Corporation, (A U.S. Company or Corporation), Seattle, WA (Washington), US (United States of America)
[Assignee Code(s): 9809]
APPL. NO.: 8-484,624
FILED: June 07, 1995 (19950607)

CROSS REFERENCE TO RELATED APPLICATION

This is application is a continuation-in-part application of U.S. patent application Ser. No. 08-249,189, filed May 24, 1994, pending, which is a continuation-in-part of U.S. patent application Ser. No. 07-969,703, filed Oct. 23, 1992, now abandoned, which is a continuation-in-part of U.S. patent application Ser. No. 07-805,723, filed on Dec. 5, 1991, now abandoned, which is a continuation-in-part of U.S. patent application Ser. No. 07-783,707, filed on Oct. 25, 1991, now abandoned.

FULL TEXT: 3795 lines

1/3/10 (Item 9 from file: 654)
DIALOG(R)File 654:US Pat.Full.

03012318
Utility
MONOCLONAL ANTIBODIES TO CD40 LIGAND, PHARMACEUTICAL COMPOSITION COMPRISING THE SAME AND HYBRIDOMAS PRODUCING THE SAME

PATENT NO.: 5,961,974
ISSUED: October 05, 1999 (19991005)
INVENTOR(s): Armitage, Richard J., Bainbridge Island, WA (Washington), US (United States of America)
Fanslow, William C., Federal Way, WA (Washington), US (United States of America)
Spriggs, Melanie K., Seattle, WA (Washington), US (United States of America)
ASSIGNEE(s): Immunex Corporation, (A U.S. Company or Corporation), Seattle, WA (Washington), US (United States of America)
[Assignee Code(s): 9809]
APPL. NO.: 8-249,189
FILED: May 24, 1994 (19940524)

CROSS REFERENCE TO RELATED APPLICATION

This application is a continuation-in-part application of U.S. patent application Ser. No. 07-969,703, filed Oct. 23, 1992, now abandoned which is a continuation-in-part of U.S. patent application Ser. No. 07-805,723, filed on Dec. 5, 1991, now abandoned which is a continuation-in-part of U.S. patent application Ser. No. 07-783,707, filed on Oct. 25, 1991 now abandoned.

FULL TEXT: 3343 lines

1/3/11 (Item 10 from file: 654)
DIALOG(R)File 654:US Pat.Full.

02994342
Utility
FUSION PROTEINS COMPRISING GP39 AND CD8

PATENT NO.: 5,945,513
ISSUED: August 31, 1999 (19990831)
INVENTOR(s): Aruffo, Alejandro, Edmonds, WA (Washington), US (United States of America)
Hollenbaugh, Diane, Seattle, WA (Washington), US (United States of America)
Ledbetter, Jeffrey A., Seattle, WA (Washington), US (United States of America)
ASSIGNEE(s): Bristol-Myers Squibb, (A U.S. Company or Corporation), Seattle, WA (Washington), US (United States of America)

APPL. NO.: 8-690,096
FILED: July 30, 1996 (19960730)

This is a division of application Ser. No. 07-940,605, filed Sep. 4, 1992 now issued as U.S. Pat. No. 5,540,926 on Jul. 30, 1996.

FULL TEXT: 1395 lines

1/3/12 (Item 11 from file: 654)
DIALOG(R)File 654:US Pat.Full.

02990817
Utility
METHOD FOR PROLONGED SUPPRESSION OF HUMORAL IMMUNE RESPONSE TO A THYMUS-DEPENDENT ANTIGEN THERAPEUTIC AGENT

PATENT NO.: 5,942,229
ISSUED: August 24, 1999 (19990824)
INVENTOR(s): Noelle, Randolph J., Cornish, NH (New Hampshire), US (United States of America)
Foy, Teresa M., Seattle, WA (Washington), US (United States of America)
ASSIGNEE(s): Trustees of Dartmouth College, (A U.S. Company or Corporation), Hanover, NH (New Hampshire), US (United States of America) [Assignee Code(s): 5682]
APPL. NO.: 8-475,873
FILED: June 07, 1995 (19950607)

This application is a continuation application of Ser. No. 08-115,990 filed on Sep. 2, 1993, now abandoned. The contents of the aforementioned application is hereby incorporated by reference.

GOVERNMENT FUNDING

The work leading to this invention may have been supported by one or more grants from the U.S. government. The U.S. government therefore may have certain rights in this invention.

FULL TEXT: 1100 lines

1/3/13 (Item 12 from file: 654)
DIALOG(R)File 654:US Pat.Full.

02980444
Utility
IN VITRO ASSAY SYSTEM FOR IDENTIFYING COMPOSITIONS USEFUL FOR STIMULATING B CELLS

PATENT NO.: 5,932,427
ISSUED: August 03, 1999 (19990803)
INVENTOR(s): Mond, James J., Potomac, MD (Maryland), US (United States of America)
Snapper, Clifford M., Kensington, MD (Maryland), US (United States of America)
ASSIGNEE(s): Henry M Jackson Foundation for the Advancement of Military Medicine, (A U.S. Company or Corporation), Rockville, MD (Maryland), US (United States of America)
APPL. NO.: 8-468,475
FILED: June 06, 1995 (19950606)

CROSS-REFERENCE TO RELATED APPLICATION

This is a continuation of prior application Ser. No. 08-315,492, filed Sep. 30, 1994, now abandoned, which is a continuation-in-part of application Ser. No. 08-150,510, filed Nov. 10, 1993, herein incorporated by reference.

GOVERNMENT INTEREST

The invention described herein may be manufactured, licensed, and used for governmental purposes without the payment of any royalties to the inventors or assignee.

FULL TEXT: 1056 lines

1/3/14 (Item 13 from file: 654)
DIALOG(R)File 654:US Pat.Full.

02963695
Utility
METHODS FOR INHIBITING AN IMMUNE RESPONSE BY BLOCKING THE GP39/CD40 AND CTLA4/CD28/B7 PATHWAYS AND COMPOSITIONS FOR USE THEREWITH

PATENT NO.: 5,916,560
ISSUED: June 29, 1999 (19990629)
INVENTOR(s): Larsen, Christian P., Atlanta, GA (Georgia), US (United States of America)
Aruffo, Alejandro A., Edmonds, WA (Washington), US (United States of America)
Hollenbaugh, Diane L., Seattle, WA (Washington), US (United States of America)
Linsley, Peter S., Seattle, WA (Washington), US (United States of America)
Ledbetter, Jeffrey A., Seattle, WA (Washington), US (United States of America)
Pearson, Thomas C., Atlanta, GA (Georgia), US (United States of America)
ASSIGNEE(s): Bristol-Myers Squibb Company, (A U.S. Company or Corporation), Pinceton, NJ (New Jersey), US (United States of America)
Emory University, (A U.S. Company or Corporation), Atlanta, GA (Georgia), US (United States of America)
[Assignee Code(s): 12419; 22921]
APPL. NO.: 8-821,400
FILED: March 20, 1997 (19970320)

This application is based on United States provisional patent application Ser. No. 60-013,751 filed on Mar. 20, 1996.

FULL TEXT: 1161 lines

1/3/15 (Item 14 from file: 654)
DIALOG(R)File 654:US Pat.Full.

02947700
Utility
METHODS OF INDUCING T CELL UNRESPONSIVENESS TO DONOR TISSUE OR ORGAN IN A RECIPIENT WITH GP39 ANTAGONISTS

PATENT NO.: 5,902,585
ISSUED: May 11, 1999 (19990511)
INVENTOR(s): Noelle, Randolph J., Cornish, NH (New Hampshire), US (United States of America)
Durie, Fiona H., Seattle, WA (Washington), US (United States

of America)
Parker, David C., Grafton, MA (Massachusettes), US (United States of America)
Appel, Michael C., Grafton, MA (Massachusettes), US (United States of America)
Phillips, Nancy E., Shrewsbury, MA (Massachusettes), US (United States of America)
Mordes, John P., Newton, MA (Massachusettes), US (United States of America)
Grenier, Dale L., Hubbardston, MA (Massachusettes), US (United States of America)
Rossini, Aldo A., Sudbury, MA (Massachusettes), US (United States of America)
ASSIGNEE(s): The Trustees of Dartmouth College, (A U.S. Company or Corporation), Hanover, NH (New Hampshire), US (United States of America)
University of Massachusetts Medical Center, (A U.S. Company or Corporation), Worcester, MA (Massachusetts), US (United States of America)
[Assignee Code(s): 5682; 22237]
APPL. NO.: 8-906,332
FILED: August 05, 1997 (19970805)

This application is a divisional of application Ser. No. 08-234,987, filed Apr. 25, 1994, now U.S. Pat. No. 5,683,693.

GOVERNMENT FUNDING

The work leading to this invention was supported in part by National Institutes of Health grants AI29544, DK41235, DK25306, DK36024, and AI26296. The U.S. government therefore may have certain rights in this invention.

FULL TEXT: 1011 lines

1/3/16 (Item 15 from file: 654)
DIALOG(R)File 654:US Pat.Full.

02944890
Utility
VACCINE DELIVERY SYSTEM

PATENT NO.: 5,900,238
ISSUED: May 04, 1999 (19990504)
INVENTOR(s): Gombotz, Wayne R., Kirkland, WA (Washington), US (United States of America)
Wee, Siow Fong, Edmonds, WA (Washington), US (United States of America)
Fanslow, III, William C., Federal Way, WA (Washington), US (United States of America)
ASSIGNEE(s): Immunex Corporation, (A U.S. Company or Corporation), Seattle, WA (Washington), US (United States of America)
[Assignee Code(s): 9809]
APPL. NO.: 8-508,229
FILED: July 27, 1995 (19950727)
FULL TEXT: 897 lines

1/3/17 (Item 16 from file: 654)
DIALOG(R)File 654:US Pat.Full.

02934624

Utility
MEMBRANE-BOUND CYTOKINE COMPOSITIONS COMPRISING GM=CSF AND METHODS OF MODULATING AN IMMUNE RESPONSE USING SAME

PATENT NO.: 5,891,432
ISSUED: April 06, 1999 (19990406)
INVENTOR(s): Hoo, William Soo, Carlsbad, CA (California), US (United States of America)
ASSIGNEE(s): The Immune Response Corporation, (A U.S. Company or Corporation), Carlsbad, CA (California), US (United States of America)
[Assignee Code(s): 32105]
APPL. NO.: 8-902,516
FILED: July 29, 1997 (19970729)
FULL TEXT: 2185 lines

1/3/18 (Item 17 from file: 654)
DIALOG(R)File 654:US Pat.Full.

02928363
Utility
DNA ENCODING TUMOR NECROSIS RELATED RECEPTOR, TR4

PATENT NO.: 5,885,800
ISSUED: March 23, 1999 (19990323)
INVENTOR(s): Emery, John, Wynnewood, PA (Pennsylvania), US (United States of America)
Tan, KB, Philadelphia, PA (Pennsylvania), US (United States of America)
Truneh, Alemseged, West Chester, PA (Pennsylvania), US (United States of America)
Young, Peter R., Lawrenceville, NJ (New Jersey), US (United States of America)
ASSIGNEE(s): Smithkline Beecham Corporation, (A U.S. Company or Corporation), Philadelphia, PA (Pennsylvania), US (United States of America)
[Assignee Code(s): 23499]
APPL. NO.: 8-794,796
FILED: February 04, 1997 (19970204)
FULL TEXT: 952 lines

1/3/19 (Item 18 from file: 654)
DIALOG(R)File 654:US Pat.Full.

02918854
Utility
MONOCLONAL ANTIBODIES SPECIFIC FOR DIFFERENT EPITOPES OF HUMAN GP39 AND METHODS FOR THEIR USE IN DIAGNOSIS AND THERAPY

PATENT NO.: 5,876,950
ISSUED: March 02, 1999 (19990302)
INVENTOR(s): Siadak, Anthony W., Seattle, WA (Washington), US (United States of America)
Hollenbaugh, Diane L., Seattle, WA (Washington), US (United States of America)
Gilliland, Lisa K., Bellevue, WA (Washington), US (United States of America)
Gordon, Marcia L., Seattle, WA (Washington), US (United States of America)
Bajorath, Jurgen, Lynnwood, WA (Washington), US (United States of America)
Aruffo, Alejandro A., Edmonds, WA (Washington), US (United

States of America)
ASSIGNEE(s): Bristol-Myers Squibb Company, (A U.S. Company or Corporation), New York, NY (New York), US (United States of America)
[Assignee Code(s): 22921]
APPL. NO.: 8-379,057
FILED: January 26, 1995 (19950126)
FULL TEXT: 3714 lines

1/3/20 (Item 19 from file: 654)
DIALOG(R)File 654:US Pat.Full.

02918627

Utility
METHODS OF INDUCING T CELL NON-RESPONSIVENESS TO TRANSPLANTED TISSUES AND OF TREATING GRAFT-VERSUS-HOST-DISEASE WITH ANTI-GP39 ANTIBODIES

PATENT NO.: 5,876,718
ISSUED: March 02, 1999 (19990302)
INVENTOR(s): Noelle, Randolph J., Cornish, NH (New Hampshire), US (United States of America)
Foy, Teresa M., Seattle, WA (Washington), US (United States of America)
Aruffo, Alejandro, Edmonds, WA (Washington), US (United States of America)
Ledbetter, Jeffrey A., Seattle, WA (Washington), US (United States of America)
ASSIGNEE(s): Bristol-Myers Squibb Company, (A U.S. Company or Corporation), New York, NY (New York), US (United States of America)
Trustees of Dartmouth College, (A U.S. Company or Corporation) , Hanover, NH (New Hampshire), US (United States of America)
[Assignee Code(s): 5682; 22921]
APPL. NO.: -49,043
FILED: March 27, 1998 (19980327)

RELATED APPLICATIONS

This application is a continuation of application Ser. No. 08-475,847, filed Jun. 7, 1995 now U.S. Pat. No. 5,747,037, in turn a continuation-in-part of application Ser. No. 08-232,929, filed Apr. 25, 1994, in turn a continuation-in-part of application Ser. No. 08-116,255, filed Sep. 2, 1993, now abandoned.

GOVERNMENT FUNDING

The work leading to this invention may have been supported by one or more grants from the U.S. government. The U.S. government therefore may have certain rights in this invention

FULL TEXT: 1782 lines

1/3/21 (Item 20 from file: 654)
DIALOG(R)File 654:US Pat.Full.

02915905

Utility
HUMAN 4-1BB RECEPTOR SPLICING VARIANT
[Polypeptides, polynucleotides for gene expression and medical diagnosis]

PATENT NO.: 5,874,240
ISSUED: February 23, 1999 (19990223)
INVENTOR(s): Ni, Jian, Rockville, MD (Maryland), US (United States of

America)
Yu, Guo-Liang, Darnestown, MD (Maryland), US (United States of America)
Gentz, Reiner, Silver Spring, MD (Maryland), US (United States of America)
Dillon, Patrick J., Gaithersburg, MD (Maryland), US (United States of America)
ASSIGNEE(s): Human Genome Sciences, Inc , (A U.S. Company or Corporation), Rockville, MD (Maryland), US (United States of America)
[Assignee Code(s): 38350]
APPL. NO.: 8-816,605
FILED: March 13, 1997 (19970313)
FULL TEXT: 2175 lines

1/3/22 (Item 21 from file: 654)
DIALOG(R)File 654:US Pat.Full.

02915751
Utility
VACCINE FOR ENHANCED PRODUCTION OF IGA ANTIBODIES
[A drug comprising at least one cytokine stimulants and a B cell activator selected from a cell surface protein and bacterial lipopolysaccharide]

PATENT NO.: 5,874,085
ISSUED: February 23, 1999 (19990223)
INVENTOR(s): Mond, James J., Jerusalem, IL (Israel)
Snapper, Clifford M., Potomac, MD (Maryland), US (United States of America)
ASSIGNEE(s): Henry M Jackson Foundation for the Advancement of Military Medicine, (A U.S. Company or Corporation), Rockville, MD (Maryland), US (United States of America)
[Assignee Code(s): 33018]
APPL. NO.: 8-400,322
FILED: March 08, 1995 (19950308)

This application is a continuation-in-part of application Ser. No. 08-315,492, filed Sep. 30, 1994, now abandoned, which is a continuation-in-part of application Ser. No. 08-150,510, filed Nov. 10, 1993, pending. Applicants specifically incorporate the prior applications by reference.

GOVERNMENT INTEREST

The invention described herein may be manufactured, licensed, and used for United States governmental purposes without the payment of any royalties to the inventors or assignee.

FULL TEXT: 912 lines

1/3/23 (Item 22 from file: 654)
DIALOG(R)File 654:US Pat.Full.

02910163
Utility
METHODS OF INDUCING T CELL UNRESPONSIVENESS TO BONE MARROW WITH GP39 ANTAGONISTS
[Allogeneic or xenogeneic bone marrow]

PATENT NO.: 5,869,049
ISSUED: February 09, 1999 (19990209)
INVENTOR(s): Noelle, Randolph J., Cornish, NH (New Hampshire), US (United

States of America)
Foy, Teresa M., Federal Way, WA (Washington), US (United States of America)
Durie, Fiona H., Seattle, WA (Washington), US (United States of America)
Parker, David C., Grafton, MA (Massachusettes), US (United States of America)
Greiner, Dale L., Hubbardston, MA (Massachusettes), US (United States of America)
Rossini, Aldo A., Sudbury, MA (Massachusettes), US (United States of America)
Mordes, John P., Newton, MA (Massachusettes), US (United States of America)
ASSIGNEE(s): Trustees of Dartmouth College, (A U.S. Company or Corporation), Hanover, NH (New Hampshire), US (United States of America)
University of Massachusetts Medical Center, (A U.S. Company or Corporation), Worcester, MA (Massachusetts), US (United States of America)
[Assignee Code(s): 5682; 22237]
APPL. NO.: 8-232,929
FILED: April 25, 1994 (19940425)

RELATED APPLICATIONS

This application is a Continuation-in-Part of U.S. patent application Ser. No. 08-116,255, filed Sep. 2, 1993 (now abandoned), the contents of which are incorporated herein by reference.

GOVERNMENT FUNDING

The work leading to this invention may have been supported by one or more grants from the U.S. government. The U.S. government therefore may have certain rights in this invention.

FULL TEXT: 1481 lines

1/3/24 (Item 23 from file: 654)
DIALOG(R)File 654:US Pat.Full.

02899098
Utility
MICE WITH TARGETED TYROSINE KINASE, LYN, DISRUPTION
[Disruption in both alleles of a gene encoding the protein]

PATENT NO.: 5,859,314
ISSUED: January 12, 1999 (19990112)
INVENTOR(s): Hibbs, Margaret L., Parkville, AU (Australia)
Dunn, Ashley R., Parkville, AU (Australia)
Graill, Dianne, Parkville, AU (Australia)
Hodgson, George, Parkville, AU (Australia)
Tarlington, David M., Parkville, AU (Australia)
Armes, Jane, Heidelberg, AU (Australia)
ASSIGNEE(s): Ludwig Institute for Cancer Research, (A U.S. Company or Corporation), New York, NY (New York), US (United States of America)
[Assignee Code(s): 28349]
EXTRA INFO: Assignment transaction [Reassigned], recorded July 26, 1999 (19990726)
APPL. NO.: 8-730,876
FILED: October 18, 1996 (19961018)
FULL TEXT: 1232 lines

1/3/25 (Item 24 from file: 654)
DIALOG(R)File 654:US Pat.Full.

02870957
Utility
TREATMENT OF T CELL MEDIATED AUTOIMMUNE DISORDERS
[Administering an antagonist to a receptor on the surface of the T cells]

PATENT NO.: 5,833,987
ISSUED: November 10, 1998 (19981110)
INVENTOR(s): Noelle, Randolph J., Cornish, NH (New Hampshire), US (United States of America)
Claassen, Eric, Pijnacker, NL (Netherlands)
ASSIGNEE(s): Nederlanse Organisatie Voor Teogepastnatuurwetenschappelijk Onderzoek TNO, (A Non-U.S. Company or Corporation), Rijswijk, NL (Netherlands)
Trustees of Dartmouth College, (A U.S. Company or Corporation), Hanover, NH (New Hampshire), US (United States of America)
[Assignee Code(s): 5263; 5682]
APPL. NO.: 8-481,735
FILED: June 07, 1995 (19950607)
FULL TEXT: 584 lines

1/3/26 (Item 25 from file: 654)
DIALOG(R)File 654:US Pat.Full.

02846718
Utility
HUMAN CARTILEGE GP39-LIKE GENE

PATENT NO.: 5,811,535
ISSUED: September 22, 1998 (19980922)
INVENTOR(s): Adamou, Julie, Exton, PA (Pennsylvania), US (United States of America)
Kirkpatrick, Robert, King of Prussia, PA (Pennsylvania), US (United States of America)
Rosenberg, Martin, Royersford, PA (Pennsylvania), US (United States of America)
ASSIGNEE(s): SmithKline Beecham Corporation, (A U.S. Company or Corporation), Philadelphia, PA (Pennsylvania), US (United States of America)
[Assignee Code(s): 23499]
APPL. NO.: 8-694,915
FILED: August 09, 1996 (19960809)
FULL TEXT: 2240 lines

1/3/27 (Item 26 from file: 654)
DIALOG(R)File 654:US Pat.Full.

02788393
Utility
RECOMBINANT ANTIBODIES FOR HUMAN THERAPY

PATENT NO.: 5,756,096
ISSUED: May 26, 1998 (19980526)
INVENTOR(s): Newman, Roland A., San Diego, CA (California), US (United States of America)
Hanna, Nabil, Olivenhain, CA (California), US (United States of America)
Raab, Ronald W., San Diego, CA (California), US (United States of America)

ASSIGNEE(s): IDEC Pharmaceuticals Corporation, (A U.S. Company or Corporation), San Diego, CA (California), US (United States of America)
[Assignee Code(s): 40498]
APPL. NO.: 8-476,237
FILED: June 07, 1995 (19950607)

FIELD OF THE INVENTION

This application is a continuation-in-part of U.S. Ser. No. 08-379,072, filed Jan. 25, 1995 (U.S. Pat. No. 5,658,570), which is a continuation of U.S. Ser. No. 07-912,292 (abandoned), filed Jul. 10, 1992, which is a continuation-in-part of Newman et al., U.S. patent application Ser. No. 07-856,281, filed Mar. 23, 1992 (abandoned), which is a continuation-in-part of U.S. patent application Ser. No. 07-735,064, filed Jul. 25, 1991 (abandoned), the whole of which, including drawings, are hereby incorporated by reference. This invention relates to recombinant antibodies useful for human therapy, and to methods for production of such antibodies.

FULL TEXT: 1809 lines

1/3/28 (Item 27 from file: 654)
DIALOG(R)File 654:US Pat.Full.

02778837

Utility
ANTI-GP39 ANTIBODIES
[Monoclonal antibodies bound to epitopes secreted by hybridomas]

PATENT NO.: 5,747,037
ISSUED: May 05, 1998 (19980505)
INVENTOR(s): Noelle, Randolph J., Cornish, NH (New Hampshire), US (United States of America)
Foy, Teresa M., Seattle, WA (Washington), US (United States of America)
Aruffo, Alejandro, Edmonds, WA (Washington), US (United States of America)
Ledbetter, Jeffrey A., Seattle, WA (Washington), US (United States of America)
ASSIGNEE(s): Bristol-Myers Squibb Company, (A U.S. Company or Corporation), New York, NY (New York), US (United States of America)
Trustees of Dartmouth College, (A U.S. Company or Corporation), Hanover, NH (New Hampshire), US (United States of America)
[Assignee Code(s): 5682; 22921]
APPL. NO.: 8-475,847
FILED: June 07, 1995 (19950607)

RELATED APPLICATIONS

This application is a Continuation-in-Part of U.S. patent application Ser. No. 08-232,929, abandoned, filed Apr. 25, 1994, and entitled "Methods for Inducing Antigen-specific T Cell Tolerance", which is a Continuation-in-Part of U.S. patent application Ser. No. 08-116,255 (abandoned in favor of U.S. Ser. No. 08-232,929), filed Sep. 2, 1993 entitled "Methods for Inducing Antigen-specific T Cell Tolerance", the entire contents of each of which are incorporated herein by reference.

GOVERNMENT FUNDING

The work leading to this invention may have been supported by one or more grants from the U.S. government. The U.S. government therefore may have certain rights in this invention.

FULL TEXT: 1676 lines

1/3/29 (Item 28 from file: 654)
DIALOG(R)File 654:US Pat.Full.

02724853

Utility
PRODUCT AND PROCESS FOR TARGETING AN IMMUNE RESPONSE
[Immunoglobulin fusion protein]

PATENT NO.: 5,698,679
ISSUED: December 16, 1997 (19971216)
INVENTOR(s): Nemazee, David A., Denver, CO (Colorado), US (United States of America)
ASSIGNEE(s): National Jewish Center for Immunology and Respiratory Medicine , (A U.S. Company or Corporation), Denver, CO (Colorado), US (United States of America)
[Assignee Code(s): 20719]
APPL. NO.: 8-309,006
FILED: September 19, 1994 (19940919)
FULL TEXT: 1615 lines

1/3/30 (Item 29 from file: 654)
DIALOG(R)File 654:US Pat.Full.

02708144

Utility
METHOD FOR INDUCING T CELL UNRESPONSIVENESS TO A TISSUE OR ORGAN GRAFT WITH ANTI-CD40 LIGAND ANTIBODY OR SOLUBLE CD40

PATENT NO.: 5,683,693
ISSUED: November 04, 1997 (19971104)
INVENTOR(s): Noelle, Randolph J., Cornish, NH (New Hampshire), US (United States of America)
Durie, Fiona H., Seattle, WA (Washington), US (United States of America)
Parker, David C., Grafton, MA (Massachusettes), US (United States of America)
Appel, Michael C., Grafton, MA (Massachusettes), US (United States of America)
Phillips, Nancy E., Shrewsbury, MA (Massachusettes), US (United States of America)
Mordes, John P., Newton, MA (Massachusettes), US (United States of America)
Grenier, Dale L., Hubbardston, MA (Massachusettes), US (United States of America)
Rossini, Aldo A., Sudbury, MA (Massachusettes), US (United States of America)
ASSIGNEE(s): Trustees of Dartmouth College, (A U.S. Company or Corporation) , Hanover, NH (New Hampshire), US (United States of America)
University of Massachusetts Medical Center, (A U.S. Company or Corporation), Worcester, MA (Massachusetts), US (United States of America)
[Assignee Code(s): 5682; 22237]
APPL. NO.: 8-234,987
FILED: April 25, 1994 (19940425)

GOVERNMENT FUNDING

The work leading to this invention was supported in part by National Institutes of Health grants AI29544, DK41235, DK25306, DK36024, and AI26296. The U.S. government therefore may have certain rights in this

invention.

FULL TEXT: 1037 lines

1/3/31 (Item 30 from file: 654)
DIALOG(R)File 654:US Pat.Full.

02697852
Utility
METHOD OF PREVENTING OR TREATING DISEASE CHARACTERIZED BY NEOPLASTIC CELLS EXPRESSING CD40
[Administering a specific antigen binding protein in a suitable buffer]

PATENT NO.: 5,674,492
ISSUED: October 07, 1997 (19971007)
INVENTOR(s): Armitage, Richard J., Bainbridge Island, WA (Washington), US (United States of America)
Fanslow, III, William C., Federal Way, WA (Washington), US (United States of America)
Longo, Dan L., Kensington, MD (Maryland), US (United States of America)
Murphy, William J., Frederick, MD (Maryland), US (United States of America)
ASSIGNEE(s): Immunex Corporation, (A U.S. Company or Corporation), Seattle, WA (Washington), US (United States of America)
The United States of America as represented by the Department of Health and Human Services, (A U.S. Government Agency), Washington, DC (District of Columbia, US (United States of America)
[Assignee Code(s): 6814; 9809]
APPL. NO.: 8-360,923
FILED: December 21, 1994 (19941221)

CROSS-REFERENCE TO RELATED APPLICATION

This application is a continuation-in-part of U.S. application Ser. No. 08-172,664, filed Dec. 23, 1993, now abandoned.

FULL TEXT: 1295 lines

1/3/32 (Item 31 from file: 654)
DIALOG(R)File 654:US Pat.Full.

02656220
Utility
EXPRESSION VECTORS ENCODING BISPECIFIC FUSION PROTEINS AND METHODS OF PRODUCING BIOLOGICALLY ACTIVE BISPECIFIC FUSION PROTEINS IN A MAMMALIAN CELL
[Single-stranded DNA]

PATENT NO.: 5,637,481
ISSUED: June 10, 1997 (19970610)
INVENTOR(s): Ledbetter, Jeffrey A., Seattle, WA (Washington), US (United States of America)
Gilliland, Lisa K., Seattle, WA (Washington), US (United States of America)
Hayden, Martha S., San Diego, CA (California), US (United States of America)
Linsley, Peter S., Seattle, WA (Washington), US (United States of America)
Bajorath, Jurgen, Everett, WA (Washington), US (United States of America)

Fell, H. Perry, Redmond, WA (Washington), US (United States of America)
ASSIGNEE(s): Bristol-Myers Squibb Company, (A U.S. Company or Corporation), New York, NY (New York), US (United States of America)
[Assignee Code(s): 22921]
APPL. NO.: 8-121,054
FILED: September 13, 1993 (19930913)

This application is a continuation-in-part of U.S. Ser. No. 08-013,420, filed Feb. 1, 1993, now abandoned the contents of which is incorporated by reference into the present application.

FULL TEXT: 2166 lines

1/3/33 (Item 32 from file: 654)
DIALOG(R)File 654:US Pat.Full.

02579330
Utility
DETECTION OF MUTATIONS IN A CD40 LIGAND GENE
[Isolating nucleic acid, amplifying nucleic acid derived from CD40 ligand gene, determining nucleotide sequence, comparing to standard, determining if protein is expressed which binds CD40]

PATENT NO.: 5,565,321
ISSUED: October 15, 1996 (19961015)
INVENTOR(s): Spriggs, Melanie K., Seattle, WA (Washington), US (United States of America)
Armitage, Richard J., Bainbridge Island, WA (Washington), US (United States of America)
Fanslow, III, William C., Federal Way, WA (Washington), US (United States of America)
ASSIGNEE(s): Immunex Corporation, (A U.S. Company or Corporation), Seattle, WA (Washington), US (United States of America)
[Assignee Code(s): 9809]
APPL. NO.: 8-184,422
FILED: January 21, 1994 (19940121)
CROSS-REFERENCE TO RELATED APPLICATION

This application is a continuation-in-part of U.S. Patent application Ser. No. 08-009,258, filed Jan. 22, 1993 now abandoned.

FULL TEXT: 1142 lines

1/3/34 (Item 33 from file: 654)
DIALOG(R)File 654:US Pat.Full.

02552628
Utility
SOLUBLE AND ITS USE IN B CELL STIMULATION
[Gp39 protein]

PATENT NO.: 5,540,926
ISSUED: July 30, 1996 (19960730)
INVENTOR(s): Aruffo, Alejandro, Edmonds, WA (Washington), US (United States of America)
Hollenbaugh, Diane, Seattle, WA (Washington), US (United States of America)
Ledbetter, Jeffrey A., Seattle, WA (Washington), US (United States of America)
ASSIGNEE(s): Bristol-Myers Squibb Company, (A U.S. Company or Corporation), Seattle, WA (Washington), US (United States of America)

[Assignee Code(s): 22921]
APPL. NO.: 7-940,605
FILED: September 04, 1992 (19920904)
FULL TEXT: 1398 lines

t s1/k/all

1/K/1 (Item 1 from file: 653)
DIALOG(R)File 653:(c) format only 2000 Knight-Ridder Info. All rts. reserv.

... T cell proliferating portion of the pre-S(2) polypeptide may be chemically coupled or **linked** to another, primary **immunogen** to form a **conjugate**. The resulting **conjugate** may then be incorporated into a **vaccine** or other inoculum as an active **immunogen** . In addition to the already described pre-S(2) region-containing inducer of T cell...

... kD polypeptide that may be used herein. That material was reported produced by cleavage of **GP39** and GP42; and GP33 and GP36 polypeptides, respectively, from HBV-infected serum using the Staphylococcus...In one embodiment, the 55 residue polypeptide is used as a carrier for the primary **immunogen** such as a polypeptide corresponding to positions 110-137 from the amino-terminus of the...

1/K/2 (Item 1 from file: 654)
DIALOG(R)File 654:(c) format only 2000 The Dialog Corp. All rts. reserv.

... subsequently administered to the individual to provide a stronger and improved immune response to the **antigen**.

The activated **antigen**-presenting dendritic cells can also be used as a **vaccine** adjuvant and can be administered prior to, concurrently with or subsequent to **antigen** administration. Moreover, the dendritic cells can be administered to the individual prior to, concurrently ... cytokines that modulate an immune response, for example a CD40 binding protein (i.e., soluble **CD40L**), or a soluble CD83 molecule. Additional useful cytokines include, but are not limited to, Interleukins...

... such as GM-CSF, granulocyte colony stimulating factor (G-CSF), or GM-CSF/IL-3 **fusion** proteins, or other cytokines such as TNF- alpha or c-kit ligand. Moreover, biologically active...
... and IL-3) will also be useful in ex vivo culture of dendritic cells. Such **fusion** proteins are known and are described in U.S. Pat. Nos. 5,199,942, 5...

... 910 and 5,073,627, each of which is incorporated herein by reference. A preferred **fusion** protein is PIXY321 as described in U.S. Pat. No. 5,199,942.

In addition cytokine or cytokines with activated, **antigen**-pulsed dendritic cells. Preferred cytokines are those that modulate an immune response, particularly cytokines selected...

... 7, 10, 12 and 15; granulocyte-macrophage colony stimulating factor, granulocyte colony stimulating factor; a **fusion** protein comprising Interleukin-3 and granulocyte-macrophage colony stimulating factor; Interferon- gamma ; TNF; TGF- beta ; flt-3 ligand; soluble **CD40 ligand** ; biologically active derivatives of these cytokines; and combinations thereof. Soluble CD83, described in U.S... site (or CD40 binding domain; variable region) of a CD40 mAb is isolated, amplified, and **linked** to DNA encoding another protein, for example a human IgG (see Verhoeyen et al., supra; see also Reichmann et al., supra). Alternatively, the **antigen**-binding site (variable region) may be either **linked**

to, or inserted into, another completely different protein (see Chaudhary et al., supra), resulting in a new protein with **antigen**-binding sites of the antibody as well as the functional activity of the completely different protein.

Similarly, the CD40 binding region (extracellular domain) of a **CD40 ligand** may be used to prepare other CD40 binding proteins. Useful forms of **CD40 ligand** are disclosed in U.S. Ser. No. 08/477,733 and U.S. Ser. No. 08/484,624, both of which were filed on Jun. 7, 1995. Additional forms of **CD40 ligand** can be prepared by methods known in the art. As for other useful CD40 binding proteins, **CD40 ligand** will bind CD40 in or near the ligand binding domain, and will be capable of oligomers will be particularly useful in preparation of CD40 binding proteins comprising an **antigen** binding domain of CD40 antibody, or an extracellular domain of a **CD40 ligand**. Certain of such oligomer-forming proteins are disclosed in U.S. Ser. No. 08/477...

...also disclosed in U.S. Ser. No. 08/446,922, filed May 18, 1995. Fc **fusion** proteins (including those that are formed with Fc muteins have decreased affinity for Fc receptors... in stimulating a certain type of immune response, administration of other cytokines along with activated, **antigen** -pulsed dendritic cells is also contemplated. Several useful cytokines (or peptide regulatory factors) are discussed...

... 7, 10, 12 and 15; granulocyte-macrophage colony stimulating factor, granulocyte colony stimulating factor; a **fusion** protein comprising Interleukin-3 and granulocyte-macrophage colony stimulating factor; Interferon- gamma, TNF, TGF- beta, flt-3 ligand and biologically active derivatives thereof. A particularly preferred cytokine is **CD40 ligand** (CD40L). A soluble form of CD40L is described in U.S. Ser. No. 08/484...

1/K/3 (Item 2 from file: 654)
DIALOG(R)File 654:(c) format only 2000 The Dialog Corp. All rts. reserv.

... which abolish the expression of these proteins. For example, naturally occurring mutations in the FAS **antigen** and its ligand cause lymphoproliferative disease (Watanabe-Fukunaga, R., et al., Nature 356:314 (1992)), perhaps reflecting a failure of programmed cell death. Mutations of the **CD40 ligand** cause an X-**linked** immunodeficiency state characterized by high levels of immunoglubulin M and low levels of immunoglobulin G...

1/K/4 (Item 3 from file: 654)
DIALOG(R)File 654:(c) format only 2000 The Dialog Corp. All rts. reserv.

... not develop any anti-PreS antibody response after repeated immunization with the PreS-TB peptide **linked** to beads.

Thus, these results clearly demonstrate that the stimulation of antibody synthesis by peptide **linked** to beads requires the activation of MHC class II-restricted helper T cells.

2.4 Mediation of the cooperation between B and T cells activated by particulate **antigen** through physical contact via the CD40/**CD40L** interaction.

To analyse the mechanisms by which these particulate **antigens** stimulate B cells, an in vitro antibody production assay using the PreS peptide mode has...

... mice were stimulated in vitro with the different PreS peptides, in either soluble form or **linked** to the ...splenocytes were unable to produce anti-PreS antibodies in response to the PreS:B peptide **linked**

to beads in the presence of the PreS:T peptide in either particulate form (FIG...
...soluble form.

To further analyze the mechanisms regulating the induction of antibodies by these peptides **linked** to 1 mu m beads, the cell cultures were stimulated with these various **immunogens** in the presence of an anti-CD40 monoclonal antibody which was shown to inhibit the binding of soluble **CD40 ligand** (**CD40L**) to soluble CD40 and to cell-surface CD40 molecule. The addition of this anti-CD40...

1/K/5 (Item 4 from file: 654)
DIALOG(R)File 654:(c) format only 2000 The Dialog Corp. All rts. reserv.

... and references cited therein: Hollenbaugh, D., Douthwright, J., McDonald, V., and Aruffo, A., "Cleavable CD40Ig **fusion** proteins and the binding to sgp39", J. Immunol. Methods (Netherlands), 188(1), p. 1-7...

... Noelle, R. J., Stamenkovic, I., Ledbetter, J. A., and Aruffo, A., "The human T cell **antigen gp39**, a member of the TNF gene family, is a ligand for the CD40 receptor: expression of a soluble form of **gp39** with ...al., "Treatment of rheumatoid arthritis with a recombinant human tumor necrosis factor receptor (P75)-Fc **fusion** protein, New England J. of Medicine, 337(3), p. 141-147(1997).

The above other...

1/K/6 (Item 5 from file: 654)
DIALOG(R)File 654:(c) format only 2000 The Dialog Corp. All rts. reserv.

... containing approximately 2000 individual clones was identified as potentially positive for binding the CD40/Fc **fusion** protein. The pool was titered and plated to provide plates containing approximately 200 colonies each...

... by the presence of an expressed gene product capable of binding to the CD40/Fc **fusion** protein.

The positive smaller pool was titered and plated to obtain individual colonies. Approximately 400

1/K/7 (Item 6 from file: 654)
DIALOG(R)File 654:(c) format only 2000 The Dialog Corp. All rts. reserv.

... are expressed on a dendritic cell. Examples include GM-CSF, IL-1, TNF, IL-4, **CD40L**, CTLA4, CD28, and FLT-3 ligand.

II. Immunostimulatory Polypeptide Complexes

A. Selection of Components of the Polypeptide Complex

An **immunogenic** polypeptide formed in accordance with the present invention is generally characterized as an isolated polypeptide **antigen** which is covalently **linked** to a dendritic cell-binding protein.

1. Polypeptide **Antigens**

As stated above, isolated polypeptide **antigens** do not generally stimulate activation of T-cells in vivo or in vitro. It is the discovery of the present invention that certain types of polypeptide **antigens**, when coupled to a dendritic cell-binding proteins, such as those discussed in Section 1...

1/K/8 (Item 7 from file: 654)
DIALOG(R)File 654:(c) format only 2000 The Dialog Corp. All rts. reserv.

OTHER REFERENCES

...80-82.

Callard, R.E. et al. (1993) "CD40 ligand and its role in X-**linked** pyper-IgM syndrome" Immunol. Today. 14:559-564.

Daniels, H.M. et al. (1989) "Spontaneous...

...infection" Lancet. 335:875-877.

Foy, T.M. et al. (1993) "In vivo CD-40-**gp39** interactions are essential for thymus-dependent humoral immunity. II. Proloned suppression of the humoral immune response by an antibody to the ligand for CD40, **gp39**" J. Exp. Med. 178:1567-1575.

Hodgkin, P.D. et al. (1990) "Separation of events...

...lymphokines" J. Immunol. 145:2025-2034.

Hollenbaugh, D. et al. (1992) "The human T cell **antigen gp39**, a member of the TNF gene family, is a ligand for the CD40 receptor: expression of a soluble form of **gp39** with B cell co- ...4321.

Itoh et al. (1991) "The polypeptide encoded by the cDNA for human cell surface **antigen** fas can mediate apoptosis" Cell. 66:223-243.

Johnson, et al. (1986) "Expression and structure...

1/K/9 (Item 8 from file: 654)
DIALOG(R)File 654:(c) format only 2000 The Dialog Corp. All rts. reserv.

...NO:9 and SEQ ID NO:10 with human cDNA as a template.

Receptor/Fc **fusion** molecules preferably are synthesized in recombinant mammalian cell culture because they are generally too large...

... synthesized by prokaryotic expression methods. Examples of suitable mammalian cells for expressing a receptor/Fc **fusion** protein include CV-1 ... transfection of the CV-1 cell line with a gene encoding Epstein-Barr virus nuclear **antigen** -I (EBNA-1) and constitutively express EBNA-1 driven from human CMV immediate-early enhancer...containing approximately 2000 individual clones was identified as potentially positive for binding the CD40/Fc **fusion** protein. The pool was titered and plated to ... by the presence of an expressed gene product capable of binding to the CD40/Fc **fusion** protein.

The positive smaller pool was titered and plated to obtain individual colonies. Approximately 400...

1/K/10 (Item 9 from file: 654)
DIALOG(R)File 654:(c) format only 2000 The Dialog Corp. All rts. reserv.

... region, which was used to ligate CD40 extracellular domain to construct a s CD40/Fc **fusion** cDNA, which was ligated into pDC406 to construct pDC406/CD40/Fc. Other suitable Fc regions...

...NO:9 and SEQ ID NO:10 with human cDNA as a template.

Receptor/Fc **fusion** molecules preferably are synthesized in recombinant mammalian cell culture because they are generally too large...

... synthesized by prokaryotic expression methods. Examples of suitable mammalian cells for expressing a receptor/Fc **fusion** protein include CV-1 cells (ATCC CCL 70) and COS-7 cells (ATCC CRL 1651...containing approximately 2000 individual clones was identified as potentially positive for binding the CD40/Fc **fusion** protein. The pool was titered and plated to provide plates containing approximately 200 colonies each...by the presence of an expressed gene product capable of binding to the CD40/Fc **fusion** protein.

The positive smaller pool was titered and plated to obtain individual colonies. Approximately 400...

1/K/11 (Item 10 from file: 654)

ABSTRACT

The present invention relates to **fusion** proteins having **gp39** protein sequences, which **fusion** proteins bind to the B cell **antigen**, CD40. More specifically, the invention relates to **fusion** proteins having **gp39** protein sequences attached to a polypeptide having an amino terminal secretory signal sequence to allow export of the **fusion** protein out of the recombinant host cell in which it is produced. The **fusion** proteins of this invention may be useful for promoting B cell proliferation.
... Ser. No. 708,075, which is incorporated by reference in its entirety herein, the soluble **gp39** proteins of the invention have a number of uses, including in vitro and in vivo...

...vitro embodiment, soluble gp39 may be used to identify or separate cells which express CD40 **antigen** and/or to assay body fluids for the presence of the CD40 **antigen** which may or may not be shed. For example, the binding of soluble **gp39** to CD40 **antigen** may be detected by directly or indirectly labeling the soluble **gp39**, for example, by incorporating radiolabel or chromogen into the soluble **gp39** protein (direct labeling) or via anti-**gp39** antibody (indirect labeling). In this manner, soluble **gp39** may be used diagnostically in vitro to identify CD40 **antigen** as expressed in tumors, malignant cells, body fluids, etc.

In related embodiments, directly or indirectly labeled soluble **gp39** may be used in vivo to image cells or tumors which express the CD40 **antigen**.

In various other in vivo embodiments, soluble **gp39** may be used to increase an immune response, for example, by acting, effectively, as a type of "adjuvant" to increase an immune response to a **vaccine** . Alternatively, soluble **gp39** may be used to increase the immune response of an immunosuppressed individual, such as a...

...malignancy, or an infant or elderly person.

In still further embodiments of the invention, soluble **gp39** may be chemically modified so that cells that it binds to are killed. Since all...

... result in suppression of the immune response. For example, a cytotoxic drug linked to soluble **gp39** may be used in vivo to cause immunosuppression in order to cross histocompatibility barriers in...

1/K/12 (Item 11 from file: 654)

...Cell Memory" J. Exp. Med. 180: 157-163.

Foy et al. (1993) "In Vivo CD40-**gp39** Interactions Are Essential for Thymus-dependent Humoral Immunity. II. Prolonged Suppression of the Hormoral Immune Response by an Antibody to the Ligand for CD40, **gp39**" J. Exp. Med. 178:1567-1575.

Harris et al. (1993) "Therapeutic antibodies--the coming of...

...Lymphokines" J. Immunol. 145(7):2025-2034.

Hollenbaugh et al. (1992) "The human T cell **antigen gp39**, a member of the TNF gene family, is a ligand for the CD40 receptor: expression of a soluble form of **gp39** with B cell co-stimulatory activity" EMBO Journal 11(12):4313-4321.

Korthauer et al. (1993) "Defective expression of T-cell **CD40 ligand** causes X-**linked** immunodeficiency with hyper-IgM" Nature 361:539-541.

Lane et al. (1992) "Activated human T cells express a ligand for the human B cell-associated **antigen** CD40 which particpates in T-cell-dependent activation of B lymphocytes" Eur. J. Immunol. 22...

... The current invention pertains to methods for inhibiting a humoral immune response to a TD **antigen** in vivo by inhibiting the ability of a Th cell to stimulate a B cell... humanized antibodies and antibody fragments are also within the scope of the invention. Alternatively, the **gp39** antagonist can be a soluble form of the **gp39** ligand CD40. Soluble fusion proteins of CD40 are also encompassed by the invention.

The humoral...

... can be a primary humoral immune response, in the case of initial exposure to an **antigen**, or a secondary humoral immune response, in the case of reexposure to a previously encountered **antigen**. For example, the methods described herein can be used to inhibit production of **antigen** -specific IgM antibodies, IgG antibodies, IgD antibodies and/or IgE antibodies. In addition, the methods...

...vivo.

One aspect of the invention provides methods for inhibiting humoral immune responses to TD **antigens**. **Antigens** embraced by the invention include **antigens** for which specific antibody production requires interaction of **gp39** with a ligand on the surface of B cells (e.g., CD40). TD **antigens** generally include proteinaceous **antigens**. In preferred embodiments of the invention, the **antigen** is a therapeutic antibody, drug, allergen or foreign cell. The methods of the present invention also are effective for inhibiting humoral immune responses to a TD **antigen** while preserving humoral immune responses to thymus-independent type II (hereafter TI-2) **antigens**.

Another aspect of the invention pertains to methods for specifically inhibiting the helper function of activated Th cells in vivo by interfering with the interaction of **gp39** with a ligand on the surface of B cells (e.g., CD40) by administering a **gp39** antagonist. According to this method, helper function of activated Th cells is inhibited in vivo... ... dependent (TD) antigens requires not only B lymphocytes, which can produce specific antibodies against an **antigen** , but also contributions from Th cells which are necessary for activation of B lymphocytes. Although... Fab or F(ab)'2 fragments, chimeric antibodies or

humanized antibodies), soluble forms of a **gp39** ligand (e.g., soluble CD40), soluble forms of a fusion protein of a **gp39** ligand (e.g., soluble CD40Ig), or pharmaceutical agents which disrupt the **gp39**-CD40 interaction.

A. Antibodies

A mammal, (e.g., a mouse, hamster, or rabbit) can be immunized with an **immunogenic** form of **gp39** protein or protein fragment (e.g., peptide fragment) which elicits an antibody response in the mammal. A cell which expresses **gp39** on its surface can also be used as the **immunogen**. Alternative **immunogens** include purified **gp39** protein or protein fragments. **gp39** can be purified from a **gp39** -expressing cell by standard purification techniques; **gp39** cDNA (Armitage et al., Nature, 357:80-82 (1992); Lederman et al., J. Exp. Med... be expressed in a host cell, e.g. bacteria or a mammalian cell line, and **gp39** protein purified. **gp39** peptides can be synthesized based upon the amino acid sequence of **gp39** (Armitage et al., Nature, 357:80-82 (1992); Lederman et al., J. Exp. Med., 175...

... al., EMBO J., 11:4313-4319 (1992)). Techniques for conferring immunogenicity on a protein include **conjugation** to carriers or other techniques well known in the art. For example, the protein can...to the hinge, CH2 and CH3 regions of C gamma 1 to form a CD40Ig **fusion** protein (see e.g., Linsley et al. (1991) J. Exp. Med. 1783:721-730; Capon ... suppressing humoral immunity against antigens which require contact-dependent helper functions delivered by Th cells. **Antigens** classically described as thymus-dependent (TD) **antigens** are encompassed by the invention. The necessity for contact-dependent "help" from Th cells can...

...cells and CD40 on B cells. As defined by the current invention, the term "TD **antigen**" is intended to encompass **antigens** which require a **gp39** -CD40 interaction between T cells and B cells for induction of a humoral immune response against the **antigen**. In general, protein **antigens** are TD **antigens**. Another form of TD **antigen** encompassed by the invention is a molecule, referred to as a hapten, linked to a...

... T cell help in order to induce humoral immune responses against the hapten.

The TD **antigen** of the invention can be administered in soluble form to a subject, e.g., injection of a soluble protein, or the TD **antigen** can be on the surface of a cell, e.g., a cell-surface protein. The TD **antigen** can be administered to a subject with a **gp39** antagonist or a subject may be exposed to a TD **antigen** environmentally, for example an allergen. In preferred embodiments, the TD **antigen** is an agent administered to a subject for therapeutic purposes. This agent can be, for example, a therapeutic antibody or other form of therapeutic drug which is a TD **antigen**. Inhibiting a humoral immune response against, for instance, a therapeutic antibody, can prolong its efficacy...when immune suppression was evident, it is possible that the local tissue concentrations of anti-**gp39** in specific sites of secondary lymphoid organs is higher and clearance rates are slower than...

... the terminal arterioles (TA) of the spleen. It is at these sites that conjugates between **gp39**-expressing T sub h and **antigen**-specific B cells were found juxtaposed, suggesting that the outer PALS is a major site...

... during primary humoral immune responses. Therefore, the PALS may be the site at which anti-**gp39** interacts with **gp39**- expressing T sub h cells to ultimately inhibit T-B interaction and subsequent Ig production...

... to KLH in CFA was also shown to be inhibited by the administration of

anti-**gp39**. Consistent with the reduction of anti-SRBC PFC by anti-**gp39**, reductions in serum antibodies titers to **antigenic** challenge were also observed. The serum titers of all anti-KLH Ig isotypes measured (IgM...

...2b, IgG sub 3, and IgE) were reduced by the treatment of mice with anti-**gp39**. The effect of anti-**gp39** administration was apparent for at least 14d after secondary challenge with **antigen**, establishing a persistent immune suppression by anti-**gp39**. Anti-**gp39**-mediated immune suppression of secondary responses to KLH is not unique to KLH, since secondary immune responses to heterologous Ig and heterologous erythrocytes were also inhibited by anti-**gp39** therapy. The anatomical distribution of **gp39** -expressing T sub h was identical to that observed upon primary immunization, however, the frequency of **gp39** -expressing Th in immune spleen was increased over that observed during primary immune responses. No **gp39**-expressing T sub h were
...method for inducing prolonged inhibition of a humoral immune response to a thymus dependent (TD) **antigen** in vivo, comprising administering a therapeutic agent which is a thymus dependent (TD) **antigen** to a subject in need of such treatment, wherein said humoral immune response is a...

... prior, subsequent, or concurrent to the administration of said therapeutic agent an amount of a **gp39** (**CD40 ligand**) antagonist selected from the group consisting of anti-**gp39** antibodies, fragments of said antibodies which specifically bind **gp39**, soluble CD40 and soluble CD40 **fusion** proteins which is sufficient to provide for prolonged inhibition of a humoral immune response to...

1/K/13 (Item 12 from file: 654)

... cells must first be activated. There are many ways to activate B cells, including cross-**linkage** of membrane Ig molecules by the **antigen** (cross-**linkage**-dependent B cell activation), direct encounter with T cells (helper T cells or helper T cell-associated molecules, such as, for example, **CD40 ligand**), or encounter with mitogens. In such encounters, the **antigen** presents epitopes recognized by the B cell's cell-surface Ig.

Because each B cell bears multiple membrane Ig molecules with identical variable regions, optimal membrane-Ig mediated cross-**linkage** activation is achieved by a high level of cross-**linkage** of the cell-surface receptors, which requires that the **antigen** present more than one copy of the epitope that the cell-surface Ig recognizes. Although many simple protein **antigens** do not have this potential, such a requirement is fulfilled by polysaccharides and other antigens **linkage** of membrane Ig can also lead to elimination or inactivation of B cells. In general...
... that retain. GM-CSF or IL-3 activity, or a combination thereof, can be independently **conjugated** to the multivalent carrier. Alternatively, GM-CSF and IL-3 can be fused together or...

...prolonged delivery of the cytokine. The complexes can be administered as a mixture with the **antigen** of a **vaccine**, or the complexes can be bound to the **antigen** of a **vaccine**.

In still another embodiment, the **vaccine** adjuvant can comprise **CD40L** , one or more cytokines other than GM-CSF, IL-3, or IFN- gamma, or a combination thereof. **CD40L** and the one or more cytokines can also be bound to the multivalent carrier.

Compositions used in a conjugate **vaccine**

To form a conjugate **vaccine**, the **antigen** of the **vaccine** and the compositions of the invention can be covalently conjugated to a multivalent carrier molecule...

... a capsular polysaccharide of a bacteria. Pneumococci, streptococci, and meningococci capsular polysaccharides are preferred.

The **antigen** is a peptide or protein specific for the disease to be **vaccinated** against.

To further optimize the humoral immune response upon administration of the vaccine, CD40 or at least one other cytokine, or a combination thereof, can be **conjugated** to the multivalent carrier.

Table III shows exemplary vaccines employing the compositions of the invention. As noted in the Table, several of the vaccines are **conjugate** vaccines. Methods of **conjugation** are well known to those of ordinary skill in the art, and include the heteroligation... multivalent carrier, i.e.:

antigen
vertical line
cytokine
vertical line
multivalent carrier

5. cytokine-antigen (**fusion** protein)
6. cytokine-antigen (**fusion** protein) bound to a multivalent carrier
7. peptide-cytokine (**fusion** protein) + antigen
8. peptide-cytokine (**fusion** protein) + antigen, with the **fusion** protein, antigen, or both bound to a multivalent carrier
9. cytokine-multivalent carrier, i...

...line

multivalent carrier — multivalent carrier

10. antibody complex (i.e., IL-3 + anti-IL-3) + **antigen**
11. antibody complex (i.e., IL-3 + anti-IL-3) + **antigen** + multivalent carrier (the cytokine, **antigen**, or both can be **conjugated** to the carrier)
12. anti-cytokine antibody; this is a neutralizing **vaccine**
13. anti-cytokine antibody + multivalent carrier; this is a neutralizing **vaccine**
14. **Vaccine** examples 1 through 13 can be further modified by the addition of **CD40L**, either admixed or bound to the multivalent carrier
15. **Vaccine** examples 1 through 14 can be further modified by the addition of one or more...

1/K/14 (Item 13 from file: 654)
DIALOG(R)File 654:(c) format only 2000 The Dialog Corp. All rts. reserv.

...J. Immunol. 139:3260 (1987)).

In another embodiment, the ligand may be a CD28Ig/CTLA4Ig **fusion** protein hybrid having a first amino acid sequence corresponding to a portion of the extracellular...

...C gamma 1.

In one embodiment of the invention, the second soluble ligand for the **gp39 antigen** may be a monoclonal antibody reactive with the **gp39 antigen**, e.g., the MR1 anti-murine monoclonal antibody or the anti-human **gp39** antibody (U.S. Pat. No. 5,474,771, issued Dec. 12, 1995).

In another embodiment of the invention, the method comprises administering to the subject a soluble **fusion** protein, the soluble **fusion** protein comprising a first binding domain and a second binding domain.

In one example, the first binding domain is a ligand which recognizes and binds the **gp39 antigen**. Examples include CD40 and monoclonal antibodies directed against **gp39** . In another example, the first binding domain is a ligand which recognizes and binds the CD40 **antigen**. Examples include **gp39** and monoclonal antibodies directed against CD40.

In one example, the second binding domain is a...

... another example, the second binding domain is a ligand which recognizes and binds the CD28 **antigen** . Examples include B7 and monoclonal antibodies directed against CD28. In another example, the second binding domain is a ligand which recognizes and binds the B7 **antigen**. Examples include CTLA4, CD28 and monoclonal antibodies directed against B7.

Soluble ligands may be administered... of (a) soluble ligands which recognize and bind any one of CTLA4, CD28, and B7 **antigens**, together with (b) soluble ligands which recognize and bind any one of **gp39** and CD40 **antigens** and an acceptable carrier. In another embodiment, these compositions comprise an effective amount of a soluble **fusion** protein comprising a first binding domain and a second binding domain, wherein the first binding domain is a ligand which recognizes and binds any one of **gp39** or CD40 **antigens** and the second binding domain is a ligand which recognizes and binds any one of CTLA4, CD28, and B7 **antigens**.

ADVANTAGES OF THE INVENTION: Despite the many advances in clinical immunosuppression, chronic vascular rejection remains...

... no effective therapy. The experiments described herein show that blocking the CD28/CTLA4/B7 and **gp39** /CD40 pathways inhibits the development of chronic transplant vasculopathy in transplanted tissues. These data show...

1/K/15 (Item 14 from file: 654)
DIALOG(R)File 654:(c) format only 2000 The Dialog Corp. All rts. reserv.

... gp39 antagonist can be an antibody directed against gp39 (e.g., a monoclonal antibody against **gp39**), a fragment or derivative of an antibody directed against gp39 (e.g., Fab or F(ab)'2 fragments, chimeric antibodies or humanized antibodies), soluble forms of a **gp39** ligand (e.g., soluble CD40), soluble forms of a fusion protein of a **gp39** ligand (e.g., soluble CD40Ig), or pharmaceutical agents which disrupt or interfere with the **gp39**-CD40 interaction.

A. Antibodies

A mammal, (e.g., a mouse, hamster, or rabbit) can be immunized with an **immunogenic** form of **gp39** protein or protein fragment (e.g., peptide fragment) which elicits an antibody response in the mammal. A cell which expresses **gp39** on its surface can also be used as the **immunogen**. Alternative **immunogens** include purified **gp39** protein or protein fragments. **gp39** can be purified from a **gp39** -expressing cell by standard purification techniques. Additionally, **gp39** cDNA (Armitage et al., Nature, 357:80-82 (1992); Lederman et al., J. Exp. Med... be expressed in a host cell, e.g., bacteria or a mammalian cell line, and **gp39** protein purified from cell cultures by standard techniques. Alternatively, **gp39** peptides can be synthesized based upon the amino acid sequence of **gp39** (disclosed in Armitage et al., Nature, 357:80-82 (1992); Lederman et al., J. Exp...

... F-moc or T-boc chemical synthesis). Techniques for conferring immunogenicity on a protein include **conjugation** to carriers or other techniques well known in the art. For example, the protein can...and CH3 regions of an immunoglobulin heavy chain, e.g. Cyl, to form a CD40Ig **fusion** protein (see e.g., Linsley et al. (1991) J. Exp. Med. 1783:721-730; Capon...

...1989) Nature 337, 525-531; and Capon U.S. Pat. No. 5,116,964). The **fusion** protein can be produced by chemical synthesis, or, preferably by recombinant DNA techniques based on...

...CD40 (Stamenkovic et al., EMBO J., 8:1403-1410 (1989)).

II. Cells for Induction of **Antigen**-Specific Tolerance

The current invention is based, at least in part, on the discovery that presentation of alloantigens to T cells by allogeneic cells in the presence of a **gp39** antagonist results in T cell ...alloantigens. Cells which are capable of inducing tolerance by this mechanism include those which present **antigen** and activate T cells by interaction with **gp39** (i.e. an interaction between **gp39** on T cells and a **gp39** ligand on the cell presenting **antigen** is necessary to deliver the appropriate signals for T cell activation to the T cell). Inhibition of the interaction of the ligand on the allogeneic or xenogeneic cell with **gp39** on recipient T cells prevents T cell activation by allo- or xenoantigens and, rather, induces T cell tolerance to the **antigens**. Interference with activation of the T cell via **gp39** may prevent the induction of costimulatory molecules on the allogeneic or xenogeneic cell, (e.g. B7 family molecules on a B cell), so that the cell delivers only an **antigenic** signal to the T cell in the absence of a costimulatory signal, thus inducing tolerance...

... is administered to a recipient subject. The allogeneic or xenogeneic cell is capable of presenting **antigen** to T cells of the recipient, and is, for example, a B lymphocyte, a "professional...In contrast, histological sections of islet allografts in the kidneys of recipients treated with anti-**gp39** mAb alone showed characteristic intense mononuclear cell inflammation and attendant islet cell destruction. In all ...

...islet morphology was uniformly consistent with streptozotocin diabetes.

EXAMPLE 2

Production and Characterization of Anti-**gp39** Antibodies

Experiment 1--Antibodies directed against human **gp39**

For induction of **antigen**-specific T cell tolerance in a human subject, it is preferable to administer an antibody directed against human **gp39**. The following methodology was used to produce mouse anti-human **gp39** monoclonal antibodies. Balb/c mice were immunized with a soluble **gp39** **fusion** protein, **gp39** -CD8, in Complete Freund's Adjuvant (CFA). Mice were subsequently challenged 6 weeks later with soluble **gp39**-CD8 in Incomplete Freund's Adjuvant (IFA). Soluble **gp39** -CD8 was given in soluble form 4 weeks after secondary immunization. Mice were then boosted...

... soluble gp39-CD8 after an additional 2 weeks. Splenocytes were fused with the NS-1 **fusion** partner on day 4 after final immunization as per standard protocols.

Clones producing anti-human...
... to become transplanted with an allogeneic or xenogeneic cell that expresses at least one donor **antigen** and also expresses **gp39**

ligand on a cell surface thereof, an amount of a **gp39** antagonist sufficient to induce T-cell non-responsiveness to said donor tissue or organ, wherein said **gp39** antagonist is selected from the group consisting of anti-**gp39** antibodies and fragments thereof that specifically bind **gp39**, soluble CD40 and soluble CD40 **fusion** proteins.

2. The method of claim 1, wherein the **gp39** antagonist is an anti-**gp39** antibody.

3. The method of claim 2, wherein the anti-**gp39** antibody is a monoclonal antibody.

4. The method of claim 2, wherein the anti-gp39...to become transplanted with an allogeneic or xenogeneic cell which expresses at least one donor **antigen** an effective amount of a **gp39** antagonist selected from the group consisting of anti- **gp39** antibodies and fragments thereof that specifically bind **gp39**, soluble CD40 and soluble CD40 **fusion** proteins, wherein the amount is effective to induce T-cell non-responsiveness to a transplanted donor tissue or organ.

14. The method of claim 13, wherein the **gp39** antagonist is an anti-**gp39** antibody.

15. The method of claim 14, wherein the anti-**gp39** antibody is a monoclonal antibody.

16. The method of claim 14, wherein the anti-gp39...

1/K/16 (Item 15 from file: 654)

... incorporated into alginate beads in a manner similar to that described herein for encapsulation of **antigens**, either alone or in conjunction with the **antigen**(s).

Exemplary immunoregulatory molecules include granulocyte-macrophage colony stimulating factor (GM-CSF), such as that described in U.S. Pat. No. 5,393,870; a **fusion** protein comprising GM-CSF and Interleukin-3 (IL-3), described in U.S. Pat. No. 5,073,627; **CD40 ligand**, described in U.S. Ser. No. 08/249,189, filed May 24, 1994; Interleukin-15 ...CD30 ligand, described in U.S. Ser. No. 98/399,415, filed Mar. 2, 1995. **Fusion** proteins comprising **antigen** and an immunoregulatory molecule will also be useful in the inventive compositions. For example, U.S. Ser. No. 08/271,875, filed Jul. 7, 1994, discloses **fusion** proteins of GM-CSF and antigens. Other cytokines may also be used to prepare such...
...antigen;
(b) forming microbeads comprising the alginate and the antigen by micronizing the alginate and **antigen** solution
(c) curing the microbeads;
(d) stabilizing the cured microbeads by contacting the microbeads with...

... the group consisting of GM-CSF, a fusion protein comprising GM-CSF and IL-3, **CD40 ligand**, IL-15, IL-16, antagonists of the interaction of CD30 and CD30 ligand, and combinations thereof.

4. The composition according to claim 1, wherein the **antigen** is a fusion protein comprising **antigen** and an immunoregulatory molecule.

5. The composition according to claim 4, wherein the immunoregulatory molecule GM-CSF, a fusion protein comprising GM-CSF and IL-3, **CD40 ligand** , IL-15, IL-16, and antagonists of the interaction of CD30 and CD30 ligand.

6. The composition according to claim 1, further comprising an **immunogenic** amount of one or more additional **antigens** encapsulated in the alginate microbead.

7. The composition according to claim 6, wherein at least one of the **antigens** is a fusion protein comprising the **antigen** and an immunoregulatory molecule.

8. The composition according to claim 7, wherein the immunoregulatory molecule...

... the group consisting of GM-CSF, a fusion protein comprising GM-CSF and IL-3, **CD40 ligand** , IL-15, IL-16, and antagonists of the interaction of CD30 and CD30 ligand.

9. A method of eliciting an immune response to an **antigen**, comprising preparing a composition according to claim 1, and administering the composition to a mucosal...

...immunoregulatory molecule encapsulated in the alginate microbead.

11. The method of claim 9, wherein the **antigen** is a **fusion** protein comprising the antigen and an immunoregulatory molecule.

12. The method of claim 9, wherein ...The method according to claim 12, wherein at least one of the antigens is a **fusion** protein comprising the antigen and an immunoregulatory molecule.

14. The method of claim 9, wherein...

1/K/17 (Item 16 from file: 654)
DIALOG(R)File 654:(c) format only 2000 The Dialog Corp. All rts. reserv.

... example, IL-4 in membrane-bound or soluble form in addition to a membrane-bound **fusion** protein that contains GM-CSF operatively fused to a heterologous membrane attachment domain. Such a cellular **vaccine** of the invention also can have GM-CSF in membrane-bound or soluble form in addition to a membrane-bound **fusion** protein that contains IL-4 operatively fused to a heterologous membrane attachment domain.

In addition, a **vaccine** of the invention can contain, if desired, a B7-1 (CD80) or B7-2 (CD86) costimulatory molecule or a CD40 or **CD40 ligand** (Chen et al., Cell 71:1093-1102 (1992); Chen et al., J. Exp. Med. 179...

... J. Immunol. 154:2794-2800 (1995), each of which are incorporated herein by reference). A **vaccine** having a B7-1 or B7-2 costimulatory molecule in addition to a membrane-bound **fusion** protein including a non-antibody immunomodulatory molecule, such as GM-CSF, IL-2, IFN- gamma...

1/K/18 (Item 17 from file: 654)
DIALOG(R)File 654:(c) format only 2000 The Dialog Corp. All rts. reserv.

... that abolish the expression of these proteins: For example, naturally occurring mutations in the FAS **antigen** and its ligand cause lymphoproliferative disease (Watanabe-Fukunaga, R., et al., Nature 356:314 (1992)), perhaps reflecting a failure of programmed cell death. Mutations of the **CD40 ligand** cause an X-**linked** immunodeficiency state characterized by high levels of immunoglubulin M and low levels of immunoglobulin G...

1/K/19 (Item 18 from file: 654)
DIALOG(R)File 654:(c) format only 2000 The Dialog Corp. All rts. reserv.

... al., "The CD40 Ligand, gp39, is Defective in Activated T Cells from Patients with X-**Linked** Hyper-IgM Syndrome," Cell 72:291-300, 1993.

Barrett, T.B. et al., "CD40 Signaling...

...Adhesion in B Cells," J. Immunol. 146:1722-1729, 1991.

Callard, R.E. et al., "**CD40 ligand** and its role in X-**linked** hyper-IgM syndrome," Immunol. Today 14;559-564,1993.

Clark, E.A. and Ledbetter, J.A., "Activation of human B cells mediated through two distinct cell surface differentiation **antigens**, Bp35 and Bp50," Proc. Natl. Acad. Sci. USA, 83:4494-4498, 1986.

Defrance, T. et... Secrete Immunoglobulin A," J. Exp. Med. 175:671-682, 1992.

DiSanto, J.P. et al., "**CD40 ligand** mutations in X-**linked** immunodeficiency with hyper-IgM," Nature 361:541-543, 1993.

Fanslow, W.C. et al., "Soluble...

... Gordon, J. et al., "Resting B Lymphocytes can be Triggered Directly through the Cdw40 (Bp50) **Antigen**," J. Immunol. 140:1425-1430, 1988.

Graf, D. et al., "Cloning of TRAP, a ligandD. et al., "The human T cell **antigen gp39**, a member of the TNF gene family, is a ligand for the CD40 receptor: expression of a soluble form of **gp39** with B cell co-stimulatory activity," EMBO J. 11:4313-4321, 1992.

Jabara, H.H...

...Med. 172:1861-1864, 1990.

Kinnon, E. and Levinsky, R., "The Molecular Basis of X-**Linked** Immunodeficiency Disease," J. Inherit. Metab. Dis. 15:674-682, 1992.

Korthauer, U. et al., "Defective expression of T-cell **CD40 ligand** causes X-**linked** immunodeficiency with hyper-IgM," Nature 361:539-541, 1883.

Lane, P. et al., "Activated human T cells express a ligand for the human B cell associated **antigen** CD40 which particpates in T cell-dependent activation of B lymphocytes," Eur. J. Immunol. 22...

... of Normal and Malignant B cell Proliferation by Monoclonal Antibody to the B cell-Specific **Antigen** BP50 (CDW4)," J. Immunol. 138:788-794, 1987.

Lederman, S. et al., "Identification of a...

... What is needed in the art are additional monoclonal antibodies reactive with different epitopes of **gp39** which can be easily used to assay for mutant forms of gp39 and for other...are replaced by alanine; and further has a poor binding avidity to a mutant of **gp39** compared to the binding avidity to wild-type **gp39** when the mutant form of **gp39** comprises glutamic acid 129, or lysine 143 replaced by alanine. The antibodies of this group also react with **gp39** by Western blot. Specific examples of monoclonal antibodies having these characteristics include those secreted by...

...156 designated TCCHB 11817.

In a further embodiment of the present invention, the monoclonal antibody, **antigen** binding fragment or recombinant binding protein

thereof is characterized by binding to a mutant form of human **gp39** with a somewhat reduced or similar binding avidity when compared to the binding avidity to wild-type **gp39** when the mutant form of **gp39** comprises glutamic acid 129, serine 131 and threonine 135, tyrosine 145, asparagine 180 or phenylalanine...

... 202 replaced by alanine, and further has a poor binding avidity to a mutant of **gp39** comprising lysine 143 replaced by alanine than to wild-type **gp39** . The antibody is further characterized by the inability to bind **gp39** in a Western blot. Specific examples of monoclonal antibodies having these characteristics are those secreted...

...designated ATCC HB 11820.

In yet another embodiment of the present invention, the monoclonal antibody, **antigen** binding fragment or recombinant binding protein thereof is characterized by its binding to mutant form of human **gp39** and to wild-type **gp39** with a similar binding avidity when the mutant comprises glutamic acid 129, serine 131 and...

... alanine. The antibody is further characterized by having poor binding avidity to a mutant human **gp39** when compared to the binding avidity to wild-type **gp39** when the mutant form comprises phenylalanine 201 and glutamic acid 202 replaced by alanine and has a somewhat reduced binding avidity to a mutant **gp39** when compared to the binding avidity to wild-type gp39 when the mutant form comprises...202 replaced by alanine. The antibody is further characterized by its ability to bind to **gp39** by Western blot. A specific example of a monoclonal antibody having these characteristics is the...
...29 designated ATCC HB 11808.

In another embodiment of the present invention, the monoclonal antibody, **antigen** binding fragment or recombinant binding protein is characterized by binding to a mutant form of human **gp39** and wild-type **gp39** with a similar binding avidity when the mutant form of **gp39** comprises glutamic acid 129, serine 131 and threonine 135, tyrosine 145, or asparagine 180 replaced...

... The antibody is also characterized by having a somewhat reduced binding avidity to a mutant **gp39** when compared to wild-type **gp39** when the mutant comprises lysine 143 replaced by alanine and also does not bind to **gp39** by Western blot. A specific example of a monoclonal antibody having these characteristics is the...

...designated HB 11823.

In still a further embodiment of the present invention, the monoclonal antibody, **antigen** binding fragment or recombinant binding protein is characterized by not being highly reactive with a mutant human **gp39** when the mutant comprises the glutamic acid at position 129, the serine at position 131...
...the monoclonal antibody is characterized as not being similarly reactive with a mutant of human **gp39** when the mutant comprises the asparagine at position 180 or the lysine at position 143...alanine. These antibodies can also be characterized by their binding or lack of binding to **gp39** by Western blot.

Each of the groups of monoclonal antibodies recognize epitopes of **gp39** and can be manipulated either chemically or by recombinant methods that generate either **antigen** binding fragments or recombinant binding proteins. Examples of **antigen** binding fragments are the Fab, (Fab') sub 2 or Fv created by enzyme digestion of...

... another embodiment of the present invention, the monoclonal antibodies or recombinant binding proteins can be **conjugated** to a detectable marker or a therapeutic agent. Examples of detectable markers include

fluorophores, radioactive...or diabetes mellitus, among others.

Further, the present invention provides methods for imaging cells expressing **gp39** on their surface in a patient which comprise administering to a patient a pharmaceutical composition including a monoclonal antibody described above **conjugated** to a detectable marker under conditions permitting the formation of antibody/**antigen** complex on the surface of the cells expressing **gp39**, and detecting the presence of the antibody/**antigen** complex as indicated by the presence of the detectable markers.
... can be accomplished by immortalizing a cell line producing antibody specific for an epitope on **gp39**. Typically, a monoclonal antibody of the present invention can be produced using well established hybridoma... Hellstrom et al. 1990, Cancer Research 50:2183.

These techniques involve the injection of an **immunogen** (e.g., cells or cellular extracts containing the **gp39 antigen** or purified **gp39** , either as native protein, a fragment containing an epitopic site, or a **fusion** protein) into an animal so as to elicit a desired immune response in that animal...

... commonly used include many mammals, e.g., mouse, rat, cow, goat, sheep, rabbit, etc. The **immunogen** is commonly presented to the animal with an adjuvant, e.g., complete Freund's adjuvent...

... peripheral blood lymphocytes, splenic lymphocytes (B-cells), or lymph node lymphocytes can be employed for **fusion** with an appropriate myeloma cell to immortalize the genes encoding monoclonal antibodies specific for **gp39**.

In the present invention, the monoclonal antibodies are partially characterized by their binding to a series of **gp39** mutants. The binding avidity (strength of binding) of the antibodies to the mutant **gp39** was compared to the binding avidity of the antibody to wild-type **gp39** . Binding avidity was characterized as poor if the comparison of the binding avidity to a particular mutant was less than 25-30% of the binding avidity to wild-type **gp39**; a weak or less profound reduction in reactivity was obtained if the binding avidity to...

...mutant was 25 to 30% to 50-55% of the binding avidity to wild-type **gp39** ; a somewhat reduced reactivity was obtained if the binding avidity to the mutant was 50...which can then be manipulated to provide for other recombinant binding proteins specific for the **gp39** epitope recognized by the parental antibody.

One such recombinant binding protein is a chimeric antibody...of a human antibody. Chimeric antibodies which are largely human in composition are substantially less **immunogenic** than murine antibodies.

Another recombinant epitope binding protein is the single chain antibody. In such...
... region from both the heavy chain and light chain of the parental antibody are covalently **linked** through a peptide **linker** such that the epitope binding region is reformed. Multivalent single chain antibodies comprising heavy and light chain variable regions specific for one or more epitopes of **gp39** can also be constructed. See EP 0 610,046 and WO 94/13806 for how...The pharmaceutical compositions of the present invention find use in vivo to inhibit the CD40/**gp39** interaction. Blocking this interaction limits both primary and secondary antibody responses to T-cell dependent **antigens** and antibody production specific for these **antigens**. Therefore, the monoclonal antibodies, **antigen** binding fragments, and recombinant binding proteins can be used to inhibit the activation of B...

...graft-versus-host disease. The compositions can also be used for imaging tumors which express **gp39** , when labeled with a detectable marker.

When **conjugated** with a therapeutic agent or as a **fusion** protein with a therapeutic agent, the monoclonal antibodies, **antigen** binding fragment or recombinant binding proteins, can also be used o target the therapeutic agent...

... The pharmaceutical compositions of the present invention find use in vivo to inhibit the CD40/**gp39** interaction. Blocking this interaction limits both primary and secondary antibody responses to T-cell dependent **antigens** and antibody production specific for these **antigens**.

This invention is illustrated in the Examples which follow. This Example section is provided to claims which follow.

EXAMPLE 1

Generation and Initial Characterization of Monoclonal Antibodies Specific for **gp39-Fusion** 1

A. Immunization

A six-to-eight-week-old female BALB/c mouse was initially immunized intraperitoneally with 30 mu g of a **gp39**-CD8 **fusion** protein (Hollenbaugh et al. 1992, EMBO J. 11:4313-4321) in a volume of 100...

... used was incomplete Freund's adjuvant. Three weeks later the mouse received an intravenous pre-**fusion** booster injection with 23 mu g of gp39 **fusion** protein in a volume of 100 mu l of phosphate buffered saline (PBS).
B. Fusion...200 mu l/well) resulting in a plating density of 243,000 total cells (pre-**fusion**) per well. Wells were fed on days 3 and 5 post **fusion** by replacement of half the supernatant with fresh hybridoma medium and assayed for anti-**gp39** specific antibody on day 8.

C. Screening

Supernatants from cell culture wells having growing cells were initially screened for reactivity with the **gp39**-CD8 **fusion** protein **immunogen** as follows. Dynatech Immulon 2 EIA plates were coated with 1 mu g/ml (100...proliferation and immunoglobulin production.

EXAMPLE 2

Generation and Initial Characterization of Monoclonal Antibodies Specific for **gp39-Fusion** 7

A. Immunization

A six-to-eight-week-old female BALB/c mouse was initially immunized subcutaneously at four sites with a total of 30 mu g of a **gp39**-CD8 **fusion** protein in complete Freund's adjuvant. Approximately two and five weeks later, this mouse was similarly injected with 30 mu g and 25 mu g, respectively, of **gp39**-CD8 except that the vehicle for **antigen** was incomplete Freund's adjuvant. Five months after initial immunization) this mouse was injected IP with 10 mu g of **fusion** protein in incomplete Freund's adjuvant. Two weeks later, the mouse received an IV pre-**fusion** booster injection of 30 mu g of **gp39** -CD8 **fusion** protein in PBS.

B. **Fusion** and Screening

Three days later, harvest, preparation, and **fusion** of the mouse spleen and lymph node cells to mouse myeloma cells was performed as for **fusion** 39-1 except that only 1 ml of PEG was used to fuse cells. The cell suspension resulting from this **fusion** was seeded into 10 96-well cell culture plates at a plating density of 183,000 total cells (pre-**fusion**) per well. Wells were fed on days 3 and 6 post fusion by

• replacement of...

1/K/20 (Item 19 from file: 654)
DIALOG(R)File 654:(c) format only 2000 The Dialog Corp. All rts. reserv.

...found that such a molecule is involved in the response of the T cell to **antigen**.

A preferred molecule on a T cell which mediates contact-dependent helper effector function is...Fab or F(ab)'2 fragments, chimeric antibodies or humanized antibodies), soluble forms of a **gp39** ligand (e.g., soluble CD40), soluble forms of a fusion protein of a **gp39** ligand (e.g., soluble CD40Ig), or pharmaceutical agents which disrupt or interfere with the **gp39**-CD40 interaction.

A. Antibodies

A mammal, (e.g., a mouse, hamster, or rabbit) can be immunized with an **immunogenic** form of **gp39** protein or protein fragment (e.g., peptide fragment) which elicits an antibody response in the mammal. A cell which expresses **gp39** on its surface can also be used as the **immunogen**. Alternative **immunogens** include purified **gp39** protein or protein fragments. **gp39** can be purified from a **gp39** -expressing cell by standard purification techniques; **gp39** cDNA (Armitage et al., Nature, 357:80-82 (1992); Lederman et al., J. Exp. Med... ... be expressed in a host cell, e.g., bacteria or a mammalian cell line, and **gp39** protein purified from the cell culture by standard techniques. **gp39** peptides can be synthesized based upon the amino acid sequence of **gp39** (disclosed in Armitage et al., Nature, 357:80-82 (1992); Lederman et al., J. Exp... F-moc or T-boc chemical synthesis). Techniques for conferring immunogenicity on a protein include **conjugation** to carriers or other techniques well known in the art. For example, the protein can...regions of an immunoglobulin heavy chain, e.g., C gamma 1, to form a CD40Ig **fusion** protein (see e.g., Linsley et al. (1991) J. Exp. Med. 1783:721-730; Capon...T cell by a cell which both presents antigen and interacts with gp39 results in **antigen** -specific T cell tolerance when the **antigen** is presented to the T cell in the presence of a **gp39** antagonist. Cells which are capable of inducing T cell tolerance by this mechanism include those which present **antigen** to a T cell and require an interaction between a **gp39** ligand on the cell and **gp39** on the T cell to deliver the necessary signals for T cell activation to the T cell. Inhibition of this interaction prevents T cell activation by the presented **antigen** and, rather, induces **antigen** -specific tolerance in the T cell. Interference with activation of the T cell via **gp39** may prevent the induction of costimulatory molecules on the **antigen** presenting cell (e.g., B7 family molecules on an **antigen** presenting cell such as a B cell) so that the **antigen** presenting cell delivers only an **antigenic** signal in the absence of a costimulatory signal, thus inducing tolerance.

Accordingly, in the methods of the invention, a cell which presents **antigen** is administered to a recipient subject. The phrase "cell which presents **antigen**" and "**antigen** presenting cell" are used interchangeably herein and ...178:1567-1575) which indicate that tolerance is not induced by the antibody in an **antigen** specific system. The two systems differ since the aGVHD model presents alloantigen already bound to **antigen** presenting cells, whereas with **antigen** specific systems the **antigen** is administered and in vivo is taken up, processed and presented by professional APC. It thus seems that anti-**gp39** may have different effects depending on the **antigen** being used and the method of presentation.

It can be concluded that anti-**gp39** may induce allospecific tolerance in both the CD4+ and CD8+ compartments of the immune system...

... immunotherapy. It is conceivable that for treatment of patients undergoing bone marrow transplants that anti-**gp39** therapy will be sufficient for induction of tolerance to the graft and prevent the induction of such consequences of transplant treatments as GVHD.

EXAMPLE 6

Production and Characterization of Anti-**gp39** Antibodies

Experiment 1--Antibodies directed against human **gp39**

For induction of **antigen** -specific T cell tolerance in a human subject, it is preferable to administer an antibody directed against human **gp39**. The following methodology was used to produce mouse anti-human **gp39** monoclonal antibodies. Balb/c mice were immunized with a soluble **gp39** fusion protein, **gp39**-CD8, in Complete Freund's Adjuvant (CFA). Mice were subsequently challenged 6 weeks later with soluble **gp39**-CD8 in Incomplete Freund's Adjuvant (IFA), Soluble ...soluble gp39-CD8 after an additional 2 weeks. Splenocytes were fused with the NS-1 **fusion** partner on day 4 after final immunization as per standard protocols.

Clones producing anti-human...

1/K/21 (Item 20 from file: 654)
DIALOG(R)File 654:(c) format only 2000 The Dialog Corp. All rts. reserv.

... that abolish the expression of these proteins. For example, naturally occurring mutations in the FAS **antigen** and its ligand cause lymphoproliferative disease (WatanabeFukunaga, R., et al., Nature, 356:314 (1992), perhaps reflecting a failure of programmed cell death. Mutations of the **CD40 ligand** cause an X-**linked** immunodeficiency state characterized by high levels of immunoglobulin M and low levels of immunoglobulin G...

1/K/22 (Item 21 from file: 654)
DIALOG(R)File 654:(c) format only 2000 The Dialog Corp. All rts. reserv.

OTHER REFERENCES

...4, pp. 1163-1170, Aug. 15, 1991.

C. Snapper et al., Multivalent, but not Divalent, **Antigen** Receptor Cross-**Linkers** Synergize with **CD40 Ligand** for Induction to Ig Synthesis and Class Switching in Normal Murine B Cells, The Journal...

...1989).

Kunimoto et al. Eur Cytokine Netw 3(4): 407-415 (1992).

Heath et al. **Vaccine** 10: 427-434 (1992).

Pecanha et al. J. Immunol 146: 833-839 (1991).

...

... assess IgA class switching in response to activation with dual combinations of a multivalent cross-**linked antigen** (dextran-**conjugated** anti-Ig antibody, or alpha delta -dex), **CD40L**, and/or LPS, plus IL-4+IL-5, in the presence or absence of TGF- beta.

FIG. 2: the percentages of mIgA sup + cells generated in response to LPS or **CD40L** , in the presence or absence of TGF- beta, with or without alpha delta -dex, IL...

... cells must first be activated. There are many ways to activate B cells, including cross-**linkage** of membrane (m) Ig molecules by the **antigen** mIg (cross-**linkage**-dependent B cell activation), direct encounter with T cells (helper T cells or helper T cell-associated molecules, such as, for example, **CD40 ligand**), or encounter with mitogens. In such encounters, the **antigen** presents epitopes recognized by the B cell's cell-surface Ig.

Because each B cell bears multiple membrane Ig molecules with identical variable regions, high level cross-**linkage** of the cell-surface receptors yields optimal activation. This cross-**linkage** requires that the **antigen** present more than one copy of the epitope that the cell-surface Ig recognizes. Although many simple protein **antigens** do not have this potential, polysaccharides and other **antigens** with repeating epitopes, such as surfaces of microbes and DNA, do. Among these more complex...

...microorganisms, such as pneumococci, streptococci, and meningococci.

There are much data to show that cross-**linkage** of membrane Ig can also lead to elimination or inactivation of B cells. In general, it is believed that certain types of receptor cross-**linkage** events, if ... al., J. Immunol. 146:833 (1991), herein incorporated by reference. Anti-IgD antibodies may be **conjugated** to dextran at a ratio ...known non-Ig-receptor activators, two are particularly suitable for the claimed invention, i.e., **CD40 ligand** and bacterial lipopolysaccharide (LPS). Of course, when the host is human, LPS is not a...

... familiar with the sources for both these receptor activators as well as the appropriate amounts. **CD40 ligand** is preferably present at about 10 mu g/ml and LPS at 20 mu g...

... The amount of this B cell receptor activator influences the preferable amount of the multivalent **antigen** receptor crosslinker. For example, the multivalent **antigen** receptor crosslinker is present at about 3 ng/ml for LPS costimulation in vitro and at about 0.3 ng/ml for **CD40 ligand** costimulation in vitro.

Such persons would also be familiar with methods to cross **link** these receptors. For example, a receptor could be inserted into membranes, such as insect cell membranes, to form a extensively cross-**linked** construct. A preferred construct is a **CD40 ligand**/insect cell membrane. Membrane bound **CD40 ligand** is prepared from sf9 insect cells infected with a recombinant CD40 ligand-containing recombinant baculovirus... J. Immunol. 129;2698), the primary site for IgA class switching in vivo.

1. Multivalent **antigen** receptor cross-**linking** of **CD40L**- or LPS-activated B cells, plus IL-4, IL -5, and TGF- beta induces a... assess IgA class switching in response to activation with dual combinations of the multivalent cross-**linked** antigen receptor, as mediated by dextran-**conjugated** anti-Ig antibody in the experimental model (alpha delta -dex), **CD40L** , and/or LPS, plus IL-4+IL-5, in the presence or absence of TGF...

...beta, IL-4+IL-5, and activation with alpha delta -dex and either LPS or **CD40L** , 10% and 12% mIgA sup + cells, respectively were generated. This is greater than a 100...

...the absence of TGF- beta.

In contrast to costimulation in the presence of a multivalent **antigen** receptor crosslinker (alpha delta -dex), dual activation with **CD40L** +LPS resulted in comparatively low levels of mIgA sup + cells.

TGF- beta was selective for induction of mIgA sup + cells following

costimulation with LPS, a multivalent **antigen** receptor crosslinker (...presence of IL-4.

2. Optimal induction of IgA class switching by either LPS- or **CD40L** -activated cells requires the independent actions of a multivalent **antigen** receptor cross-**linker**, IL-4, IL-5, and TGF- beta

To determine the relative requirements of the various...

... switching, the percentages of mIgA sup + cells generated in response to stimulation by LPS or **CD40L**, in the presence or absence of TGF- beta, with or without a multivalent **antigen** receptor crosslinker (alpha delta -dex), IL-4, and/or IL-5 were measured (FIG. 2...

... these percentages did not exceed 2%. Only in the combined presence of a multivalent cross-**linked antigen** receptor (alpha delta -dex), IL-4, IL-5, and TGF- beta were relatively large percentages of mIgA sup + cells generated upon activation with either LPS or **CD40L** (11.0% and 12.3%, respectively).

3. TGF- ...with selective stimulation of IgA secretion

Costimulation of B cells with either LPS+ a multivalent **antigen** receptor crosslinker (alpha delta -dex), or **CD40L**+ alpha delta -dex, in the presence of IL-4, IL-5, and TGF- beta, stimulated...the percentage of mIgA sup + cells generated in response to activation with either LPS or **CD40L** in the presence of a multivalent cross-**linked antigen** receptor (alpha delta -dex), IL-4, IL-5, and TGF- beta. Optimal inhibition of IgA...

...ml of IFN- gamma representing an difference 4-fold and difference 6-fold suppression for **CD40L** - and LPS-activated cells, respectively. A significant reduction in mIgA sup + cells was observed with as little as 1-3 U/ml of IFN- gamma.

7. The activity of **CD40 ligand**

Published data suggests that the activity of **CD40 ligand** may depend on the absolute amount of this protein expressed on the cell surface. In... all B cells. Thus, the in vitro model employing anti-Ig-dextran acts to cross-**link** all antigen receptors. However, this response is not desired in vivo. In a patient, the...The multivalent cross-linked antigen receptor can also serve as the carrier molecule for CD40 **ligand** and TGF- beta, IL-4, and either IL-5 or IL-2. Other **vaccine** variations will be apparent to one of skill in the art.

This application encompasses the...
...prolonged delivery of the cytokine. The complexes can be administered as a mixture with the **antigen** of a **vaccine**, or the complexes can be bound to the **antigen** of a **vaccine**.

In still another embodiment, the **vaccine** adjuvant can comprise **CD40 ligand**, one or more cytokines other than GM-CSF, IL-3, or IFN- gamma, or a combination thereof. **CD40L** and the one or more cytokines can also be bound to the multivalent carrier.

3. Compositions used in a conjugate **vaccine**

To form a conjugate **vaccine**, the **antigen** of the **vaccine** and the compositions of the invention can be covalently conjugated to a multivalent carrier molecule...

... a capsular polysaccharide of a bacteria. Pneumococci, streptococci, and meningococci capsular polysaccharides are preferred.

The **antigen** is a peptide or protein specific for the disease to be vaccinated against.

To further...
... invention. As noted in the Table, several of the vaccines are conjugate

vaccines. Methods of **conjugation** are well known to those of ordinary skill in the art, and include the heteroligation...CRC Press, Boston (1991); and Brenkeley et al., "Brief Survey of Methods for Preparing Protein **Conjugates** With Dyes, Haptens and Cross-**Linking** Agents," Bioconjugate Chemistry, 3, No. 1 (January 1992), specifically incorporated by reference.

The unexpected effect...

...only and are not intended to be limiting.

EXAMPLE 1

This example shows that multivalent **antigen** receptor cross-**linking** of **CD40L** - or LPS-activated B cells, plus IL-4, IL-5, and TGF- beta induces a...the percentage of mIgA sup + cells generated in response to activation with either LPS or **CD40 ligand** in the presence of alpha delta -dex, IL-4, IL-5 and TGF- beta. Optimal...

...ml of IFN- gamma representing an difference 4-fold and difference 6-fold suppression for **CD40 ligand** - and LPS-activated B cells, respectively. A significant reduction in mIgA sup + cells was observed...

...little as 1-3 U/ml of IFN- gamma.

EXAMPLE 6

This example provides exemplary **vaccines** and **vaccine** adjuvants employing the compositions of the invention.

Table II shows exemplary **vaccines** employing the compositions of the invention. As noted in the Table, several of the **vaccines** are **conjugate vaccines**. Methods of **conjugation** are well known to those of ordinary skill in the art, and include the heteroligationS. S., Chemistry of Protein **Conjugates** and Crosslinking, CRC Press, Boston (1991); and Brenkeley et al., "Brief Survey of Methods for...adjuvants, etc.

2. cytokine + antigen + multivalent carrier; the cytokine, antigen, or both can be directly **conjugated** to the carrier, i.e.:
   [See structure in original document]
   [See structure in original document]
3. cytokine-antigen; direct conjugation via covalent bonding
4. cytokine-antigen (**covalent** bonding) bound to a multivalent carrier, i.e.:
   [See structure in original document]
5. cytokine-antigen (**fusion** protein)
6. cytokine-antigen (**fusion** protein) bound to a multivalent carrier
7. peptide-cytokine (**fusion** protein) + antigen
8. peptide-cytokine (**fusion** protein) + antigen, with the **fusion** protein, **antigen**, or both bound to a multivalent carrier
9. cytokine-multivalent carrier, i.e.:
   [See structure in original document]
10. antibody complex (i.e., cytokine + cytokine) + **antigen**
11. antibody complex (i.e., cytokine + cytokine) + **antigen** + multivalent carrier (the cytokine, **antigen**, or both can be **conjugated** to the carrier)
12. anti-cytokine antibody; this is a neutralizing **vaccine**
13. anti-cytokine antibody + multivalent carrier; this is a neutralizing **vaccine**
14. **Vaccine** examples 1 through 13 can be further modified by the addition of **CD40L**, either admixed or bound to the multivalent carrier
15. **Vaccine** examples 1 through 14 can be further modified by the

addition of one or more...In sum, the experimental results described above show that B cells activated with a multivalent **antigen** receptor cross-**linker**, as for example dextran-**conjugated** anti-Ig antibody in the experimental model, and either **CD40 ligand** or LPS, in the presence of the cytokines TGF- beta, IL-4, and either IL... We claim:

1. A **vaccine** comprising:
the **antigen** of the **vaccine** in multivalent form;
at least one cytokine which stimulates IgA class switching; and
a B cell activator selected from the group consisting of **CD40 ligand** and LPS,
wherein said **vaccine** stimulates IgA antibody class switching.

2. The **vaccine** of claim 1, wherein the at least one cytokine is selected from the group consisting of TGF- beta, IL-2, IL-4, and IL-5.

3. The **vaccine** of claim 2 wherein the at least one cytokine is TGF-beta.

4. The **vaccine** of claim 3 further comprising IL-4 and IL-5.

5. The **vaccine** of claim 3 further comprising IL-4 and IL-2.

6. The **vaccine** of claim 1 wherein the antigen in multivalent form comprises multiple copies of at least one epitope covalently **conjugated** to a multivalent carrier selected from the group consisting of dextran and bacterial capsular polysaccharide...

1/K/23 (Item 22 from file: 654)

...found that such a molecule is involved in the response of the T cell to **antigen**.

A preferred molecule on a T cell which mediates contact-dependent helper effector function is...Fab or F(ab)'2 fragments, chimeric antibodies or humanized antibodies), soluble forms of a **gp39** ligand (e.g., soluble CD40), soluble forms of a fusion protein of a **gp39** ligand (e.g., soluble CD40Ig), or pharmaceutical agents which disrupt or interfere with the **gp39**-CD40 interaction.

A. Antibodies

A mammal, (e.g., a mouse, hamster, or rabbit) can be immunized with an **immunogenic** form of **gp39** protein or protein fragment (e.g., peptide fragment) which elicits an antibody response in the mammal. A cell which expresses **gp39** on its surface can also be used as the **immunogen**. Alternative **immunogens** include purified **gp39** protein or protein fragments. **gp39** can be purified from a **gp39** -expressing cell by standard purification techniques; **gp39** cDNA (Armitage et al., Nature, 357:80-82 (1992); Lederman et al., J. Exp. Med... ... be expressed in a host cell, e.g., bacteria or a mammalian cell line, and **gp39** protein purified from the cell culture by standard techniques. **gp39** peptides can be synthesized based upon the amino acid sequence of **gp39** (disclosed in Armitage et al., Nature, 357:80-82 (1992); Lederman et al., J. Exp... F-moc or T-boc chemical synthesis). Techniques for conferring immunogenicity on a protein include **conjugation** to carriers or other techniques well known in the art. For example, the protein can...regions of an immunoglobulin heavy chain, e.g., C gamma 1, to form a CD40Ig **fusion** protein (see e.g., Linsley et al. (1991) J. Exp. Med. 1783:721-730; Capon...

... T cell by a cell which both presents antigen and interacts with gp39 results in **antigen**-specific T cell tolerance when the **antigen**

is presented to the T cell in the presence of a **gp39** antagonist. Cells which are capable of inducing T cell tolerance by this mechanism include those which present **antigen** to a T cell and require an interaction between a **gp39** ligand on the cell and **gp39** on the T cell to deliver the necessary signals for T cell activation to the T cell. Inhibition of this interaction prevents T cell activation by the presented **antigen** and, rather, induces **antigen** -specific tolerance in the T cell. Interference with activation of the T cell via **gp39** may prevent the induction of costimulatory molecules on the **antigen** presenting cell (e.g., B7 family molecules on an **antigen** presenting cell such as a B cell) so that the **antigen** presenting cell delivers only an **antigenic** signal in the absence of a costimulatory signal, thus inducing tolerance.

Accordingly, in the methods of the invention, a cell which presents **antigen** is administered to a recipient subject. The phrase "cell which presents **antigen**" and "**antigen** presenting cell" are used interchangeably herein and are intended to encompass cells which can present... 178:1567-1575) which indicate that tolerance is not induced by the antibody in an **antigen** specific system. The two systems differ since the aGVHD model presents alloantigen already bound to **antigen** presenting cells, whereas with **antigen** specific systems the **antigen** is administered and in vivo is taken up, processed and presented by professional APC. It thus seems that anti-**gp39** may have different effects depending on the **antigen** being used and the method of presentation.

It can be concluded that anti-**gp39** may induce allospecific tolerance in both the CD4+ and CD8+ compartments of the immune system undergoing bone marrow transplants that anti-**gp39** therapy will be sufficient for induction of tolerance to the graft and prevent the induction of such consequences of transplant treatments as GVHD.

EXAMPLE 6

Production and Characterization of Anti-**gp39** Antibodies

Experiment 1--Antibodies directed against human **gp39**

For induction of **antigen** -specific T cell tolerance in a human subject, it is preferable to administer an antibody directed against human **gp39**. The following methodology was used to produce mouse anti-human **gp39** monoclonal antibodies. Balb/c mice were immunized with a soluble **gp39** fusion protein, **gp39**-CD8, in Complete Freund's Adjuvant (CFA). Mice were subsequently challenged 6 weeks later with soluble **gp39** -CD8 in Incomplete Freund's Adjuvant (IFA). Soluble gp39-CD8 was given in soluble form...

... soluble gp39-CD8 after an additional 2 weeks. Splenocytes were fused with the NS-1 **fusion** partner on day 4 after final immunization as per standard protocols.

Clones producing anti-human...

1/K/24 (Item 23 from file: 654)
DIALOG(R)File 654:(c) format only 2000 The Dialog Corp. All rts. reserv.

...R. L., Noelle, R. J., Ledbetter, J. A., Francke, U. and Ochs, H. D. "The **CD40 ligand**, **gp39**, is defective in activated T cells from patients with X-**linked** hyper-IgM syndrome" Cell, 1993 72 291-300
Banchereau, J., Bazan, F., Blanchard, D., Briere...

...J. P., van Kooten, C., Liu, Y. J., Rousset, F. and Saeland, S. "The CD40 **antigen** and its ligand" Annu. Rev. Immunol., 1994 12 881-922.
Blank, U., Ra, C., Miller...

1/K/25 (Item 24 from file: 654)
DIALOG(R)File 654:(c) format only 2000 The Dialog Corp. All rts. reserv.

... the methods of the invention include multiple sclerosis, EAE, diabetes type I, oophoritis, and thyroiditis.

gp39 ANTAGONISTS

According to the methods of the invention, a gp39 antagonist is administered to a...

...a gp39 ligand (e.g., soluble CD40), soluble forms of a fusion protein of a **gp39** ligand (e.g., soluble CD40Ig), or pharmaceutical agents which disrupt or interfere with the **gp39**-CD40 interaction.

A. Antibodies

To prepare anti-**gp39** antibodies, a mammal (e.g., a mouse, hamster, or rabbit) can be immunized with an **immunogenic** form of **gp39** protein or protein fragment (e.g., peptide fragment) which elicits an antibody response in the mammal. A cell which expresses **gp39** on its surface can also be used as the **immunogen**. Alternative **immunogens** include purified **gp39** protein or protein fragments. **gp39** can be purified from a **gp39**-expressing cell by standard purification techniques, e.g., **gp39** cDNA (Armitage et al., Nature, 357:80-82 (1992); Lederman et al., J. Exp. Med...

... be expressed in a host cell, e.g., bacteria or a mammalian cell line, and **gp39** protein purified from the cell culture by standard techniques. **gp39** peptides can be synthesized based upon the amino acid sequence of **gp39** (disclosed in Armitage et al., Nature, 357:80-82 (1992); Lederman et al., J. Exp...

... F-moc or T-boc chemical synthesis). Techniques for conferring immunogenicity on a protein include **conjugation** to carriers or other techniques well known in the art. For example, the protein can...

1/K/26 (Item 25 from file: 654)
DIALOG(R)File 654:(c) format only 2000 The Dialog Corp. All rts. reserv.

...uses it would be desirable to be able to delete the Fc part after the **fusion** protein has been expressed, detected and purified in the advantageous manner described. This is the...

... proves to be a hindrance to use in therapy and diagnosis, for example when the **fusion** protein is to be used as **antigen** for immunizations. In drug discovery, for example, human proteins, such as, shIL5- alpha has been...

...270:16, pp 9459-9471 (1995).

Thus, this invention also relates to genetically engineered soluble **fusion** proteins comprised from HC **gp39**-L, or a portion thereof, and of various portions of the constant regions of heavy...

1/K/27 (Item 26 from file: 654)
DIALOG(R)File 654:(c) format only 2000 The Dialog Corp. All rts. reserv.

... soluble receptor proteins to TNF-alpha, monoclonal antibodies to IL2 receptor, monoclonal antibodies and receptor **fusion** proteins which antagonize the CD40/**gp39** interaction and CTLA 4-Ig in monoclonal antibodies which antagonize the B7/CD28 interaction. Also...

... Leflunomide, Tenidap, RS-61443 (Mycophenolate Mofetil), Surenyl (sodium Hyaluronate), anti-TCR (V beta 17) peptide **vaccine** , Anerva X (anti-MHC **vaccine**), and extracorporeal protein A immunoabsorbants or combinations thereof. Additionally, the subject antibody may be administered...

1/K/28 (Item 27 from file: 654)
DIALOG(R)File 654:(c) format only 2000 The Dialog Corp. All rts. reserv.

...found that such a molecule is involved in the response of the T cell to **antigen**.

A preferred molecule on a T cell which mediates contact-dependent helper effector function is...Fab or F(ab)'2 fragments, chimeric antibodies or humanized antibodies), soluble forms of a **gp39** ligand (e.g., soluble CD40), soluble forms of a fusion protein of a **gp39** ligand (e.g., soluble CD40Ig), or pharmaceutical agents which disrupt or interfere with the **gp39**-CD40 interaction.

A. Antibodies

A mammal, (e.g., a mouse, hamster, or rabbit) can be immunized with an **immunogenic** form of **gp39** protein or protein fragment (e.g., peptide fragment) which elicits an antibody response in the mammal. A cell which expresses **gp39** on its surface can also be used as the **immunogen**. Alternative **immunogens** include purified **gp39** protein or protein fragments. **gp39** can be purified from a **gp39** -expressing cell by standard purification techniques; **gp39** cDNA (Armitage et al., Nature, 357:80-82 (1992); Lederman et al., J. Exp. Med... ... be expressed in a host cell, e.g., bacteria or a mammalian cell line, and **gp39** protein purified from the cell culture by standard techniques. **gp39** peptides can be synthesized based upon the amino acid sequence of **gp39** (disclosed in Armitage et al., Nature, 357:80-82 (1992); Lederman et al., J. Exp... F-moc or T-boc chemical synthesis). Techniques for conferring immunogenicity on a protein include **conjugation** to carriers or other techniques well known in the art. For example, the protein can...regions of an immunoglobulin heavy chain, e.g., C gamma 1, to form a CD40Ig **fusion** protein (see e.g., Linsley et al. (1991) J. Exp. Med. 1783:721-730; Capon cell by a cell which both presents antigen and interacts with gp39 results in **antigen**-specific T cell tolerance when the **antigen** is presented to the T cell in the presence of a **gp39** antagonist. Cells which are capable of inducing T cell tolerance by this mechanism include those which present **antigen** to a T cell and require an interaction between a **gp39** ligand on the cell and **gp39** on the T cell to deliver the necessary signals for T cell activation to the T cell. Inhibition of this interaction prevents T cell activation by the presented **antigen** and, rather, induces **antigen** -specific tolerance in the T cell. Interference with activation of the T cell via **gp39** may prevent the induction of costimulatory molecules on the **antigen** presenting cell (e.g., B7 family molecules on an **antigen** presenting cell such as a B cell) so that the **antigen** presenting cell delivers only an **antigenic** signal in the absence of a costimulatory signal, thus inducing tolerance.

Accordingly, in the methods of the invention, a cell which presents **antigen** is administered to a recipient subject. The phrase "cell which presents **antigen**" and "**antigen** presenting cell" are used interchangeably herein and are intended ...178:1567-1575) which indicate that tolerance is not induced by the antibody in an **antigen** specific system. The two systems differ since the aGVHD model presents alloantigen already bound to **antigen** presenting cells, whereas with **antigen** specific systems the **antigen** is administered and in vivo is taken up, processed and presented by professional APC. It thus seems that anti-**gp39** may have different effects depending on the **antigen** being

used and the method of presentation.

It can be concluded that anti-**gp39** may induce allospecific tolerance in both the CD4+ and CD8+ compartments of the immune system...

... immunotherapy. It is conceivable that for treatment of patients undergoing bone marrow transplants that anti-**gp39** therapy will be sufficient for induction of tolerance to the graft and prevent the induction of such consequences of transplant treatments as GVHD.

EXAMPLE 6

Production and Characterization of Anti-**gp39** Antibodies

Experiment 1--Antibodies directed against human **gp39**

For induction of **antigen** -specific T cell tolerance in a human subject, it is preferable to administer an antibody directed against human **gp39**. The following methodology was used to produce mouse anti-human **gp39** monoclonal antibodies. Balb/c mice were immunized with a soluble **gp39** fusion protein, **gp39**-CD8, in Complete Freund's Adjuvant (CFA). Mice were subsequently challenged 6 weeks later with soluble **gp39** -CD8 in Incomplete Freund's Adjuvant (IFA). Soluble gp39-CD8 was given in soluble form... soluble gp39-CD8 after an additional 2 weeks. Splenocytes were fused with the NS-1 **fusion** partner on day 4 after final immunization as per standard protocols.

Clones producing anti-human...

1/K/29 (Item 28 from file: 654)
DIALOG(R)File 654:(c) format only 2000 The Dialog Corp. All rts. reserv.

... that T cell. Anergy refers to the diminished reactivity by a T cell to an **antigen**. Effector molecules involved in T cell activation include, but are not limited to, B7, B7-2, CD28, CD40 and the **CD40 ligand** . APCs having B7 or B7-2, and CD40 are capable of activating T cells by binding to CD28 and **CD40 ligand**, respectively, on the surface of a T cell. Such APCs are referred to as professional...

...activated). Such APCs are referred to as non-professional APCs.

Preferred professional APC-specific immunoglobulin **fusion** proteins of the present invention are capable of binding to an **antigen** on the surface of dendritic cells, macrophages and B cells that express B7, B7-2 and CD40. Preferred non-professional APC-specific immunoglobulin **fusion** protein of the present invention are capable of binding to an antigen on the surface...

1/K/30 (Item 29 from file: 654)
DIALOG(R)File 654:(c) format only 2000 The Dialog Corp. All rts. reserv.

... gp39 antagonist can be an antibody directed against gp39 (e.g., a monoclonal antibody against **gp39**), a fragment or derivative of an antibody directed against gp39 (e.g., Fab or F(ab)'2 fragments, chimeric antibodies or humanized antibodies), soluble forms of a **gp39** ligand (e.g., soluble CD40), soluble forms of a fusion protein of a **gp39** ligand (e.g., soluble CD40Ig), or pharmaceutical agents which disrupt or interfere with the **gp39**-CD40 interaction.

A. Antibodies

A mammal, (e.g., a mouse, hamster, or rabbit) can be immunized with an **immunogenic** form of **gp39** protein or protein fragment (e.g., peptide fragment) which elicits an antibody response in the mammal. A cell

which expresses **gp39** on its surface can also be used as the **immunogen**. Alternative **immunogens** include purified **gp39** protein or protein fragments. **gp39** can be purified from a **gp39** -expressing cell by standard purification techniques. Additionally, **gp39** cDNA (Armitage et al., Nature, 357:80-82 (1992); Lederman et al., J. Exp. Med... be expressed in a host cell, e.g., bacteria or a mammalian cell line, and **gp39** protein purified from cell cultures by standard techniques. Alternatively, **gp39** peptides can be synthesized based upon the amino acid sequence of **gp39** (disclosed in Armitage et al., Nature, 357:80-82 (1992); Lederman et al., J. Exp...

... F-moc or T-boc chemical synthesis). Techniques for conferring immunogenicity on a protein include **conjugation** to carriers or other techniques well known in the art. For example, the protein can...regions of an immunoglobulin heavy chain, e.g. C gamma 1, to form a CD40Ig **fusion** protein (see e.g., Linsley et al. (1991) J. Exp. Med. 1783:721-730; Capon...

...1989) Nature 337, 525-531; and Capon U.S. Pat. No. 5,116,964). The **fusion** protein can be produced by chemical synthesis, or, preferably by recombinant DNA techniques based on...

...CD40 (Stamenkovic et al., EMBO J., 8:1403-1410 (1989)).

II. Cells for Induction of **Antigen**-Specific Tolerance

The current invention is based, at least in part, on the discovery that presentation of alloantigens to T cells by allogeneic cells in the presence of a **gp39** antagonist results ... alloantigens. Cells which are capable of inducing tolerance by this mechanism include those which present **antigen** and activate T cells by interaction with **gp39** (i.e. an interaction between **gp39** on T cells and a **gp39** ligand on the cell presenting **antigen** is necessary to deliver the appropriate signals for T cell activation to the T cell). Inhibition of the interaction of the ligand on the allogeneic or xenogeneic cell with **gp39** on recipient T cells prevents T cell activation by allo- or xenoantigens and, rather, induces T cell tolerance to the **antigens**. Interference with activation of the T cell via **gp39** may prevent the induction of costimulatory molecules on the allogeneic or xenogeneic cell, (e.g. B7 family molecules on a B cell), so that the cell delivers only an **antigenic** signal to the T cell in the absence of a costimulatory signal, thus inducing tolerance...

... is administered to a recipient subject. The allogeneic or xenogeneic cell is capable of presenting **antigen** to T cells of the recipient, and is, for example, a B lymphocyte, a "professional...In contrast, histological sections of islet allografts in the kidneys of recipients treated with anti-**gp39** mAb alone showed characteristic intense mononuclear cell inflammation and attendant islet cell destruction. In all ...

...islet morphology was uniformly consistent with streptozotocin diabetes.

EXAMPLE 2

Production and Characterization of Anti-**gp39** Antibodies

Experiment 1--Antibodies directed against human **gp39**

For induction of **antigen** -specific T cell tolerance in a human subject, it is preferable to administer an antibody directed against human **gp39**. The following methodology was used to produce mouse anti-human **gp39** monoclonal antibodies. Balb/c mice were immunized with a soluble **gp39** **fusion** protein, **gp39** -CD8, in Complete Freund's Adjuvant (CFA). Mice were subsequently challenged 6 weeks later with soluble **gp39**-CD8 in Incomplete Freund's Adjuvant (IFA). Soluble

gp39 -CD8 was given in soluble form 4 weeks after secondary immunization. Mice were then boosted...

... soluble gp39-CD8 after an additional 2 weeks. Splenocytes were fused with the NS-1 **fusion** partner on day 4 after final immunization as per standard protocols.

Clones producing anti-human...
...to the recipient
a) an allogeneic or xenogeneic cell which expresses at least one donor **antigen** and which has a ligand on a cell surface which interacts with a **gp39** molecule (**CD40 ligand**); and
b) an antagonist of the **gp39** molecule which inhibits interaction of the ligand with the **gp39** molecule, such that non-responsiveness of the T cell to the donor tissue or organ is induced, wherein said **gp39** antagonist is selected from the group consisting of anti-**gp39** antibodies, fragments thereof that specifically bind **gp39**, soluble CD40 and soluble CD40 **fusion** proteins.

2. The method of claim 1, wherein the antagonist is an anti-gp39 antibody ...of such treatment:
a) an allogeneic or xenogeneic cell which expresses at least one donor **antigen**;
b) a **gp39** antagonist selected from the group consisting of anti-**gp39** (**CD40 ligand**) antibodies, fragments thereof that specifically bind **gp39**, soluble CD40 and soluble CD40 **fusion** proteins; and
c) donor pancreatic islet cells.

15. The method of claim 14, wherein the anti-**gp39** antibody is a monoclonal antibody.

16. The method of claim 14, wherein the anti-**gp39** antibody is an anti-human **gp39** antibody.

17. The method of claim 15, wherein the monoclonal antibody is MR1 (ATCC Accession...

1/K/31 (Item 30 from file: 654)

...a 19 amino acid secretory signal peptide comprising predominantly hydrophobic amino acids. This cell surface **antigen** has been shown to play an important role in B-cell proliferation and differentiation. A...

... surface of monocytic and epithelial cells, and on some epithelial carcinomas (E. A. Clark, Tissue **Antigens** 36:33; 1990).

Activated CD4+ T cells express high levels of a ligand for CD40 (**CD40L**). Human **CD40L** , a membrane-bound glycoprotein, has recently been cloned from peripheral blood T-cells as described...

... 23, 1992, the disclosure of which is incorporated by reference herein. The cloning of murine **CD40L** is described in Armitage et al., Nature 357:80, 1992. **CD40L** induces B-cell proliferation in the absence of any co-stimulus, and can also induce...

...CD40 are known in the art (see, for example, the sections dedicated to B cell **antigens** in LEUKOCYTE TYPING III; ...to exert costimulatory signals on normal B cells, resulting in proliferative and differentiation responses. Similarly, **CD40L** exerts protein stimulatory or costimulatory signals to normal B cells.

It has been observed that cross-**linking** of surface IgM on some B cell lymphoma lines exerts inhibitory signals to the lymphoma...

... proteins) that specifically bind CD40 (referred to as a CD40 binding protein) in a non-**covalent** interaction based upon the proper conformation of the CD40 binding protein and CD40 itself. For example, a CD40 binding protein can comprise an extracellular region of a **CD40 ligand** . In other cases, a CD40 binding protein can comprise an antibody that binds CD40 through an **antigen** binding region. Additional CD40 binding proteins can be prepared through recombinant methods, by preparation of **fusion** proteins comprising a CD40 binding region (or domain) from a **CD40 ligand**, or an antibody to CD40, with a second protein, for example, a human immunoglobulin Fc domain.

CD40

Human CD40 **antigen** (CD40) is a peptide of 277 amino acids having a molecular weight of 30,600...binding site (or CD40binding domain; variable region) of a CD40 mAb is isolated, amplified, and **linked** to DNA encoding another protein, for example a human IgG (see Verhoeyen et al., supra...

... also Reichmann et al., supra). Alternatively, the antigen-binding site (variable region) may be either **linked** to, or inserted into, another completely different protein (see Chaudhary et al., supra), resulting in a new protein with **antigen**-binding sites of the antibody as well as the functional activity of the completely different...

...the context of the present invention. Similarly, the CD40 binding region (extracellular domain) of a **CD40 ligand** may be used to prepare other CD40 binding proteins. DNA sequences that encode proteins or...

... that form oligomers will be particularly useful in preparation of CD40 binding proteins comprising an **antigen** binding domain of CD40 antibody, or an extracellular domain of a **CD40 ligand**. Certain of such oligomer-forming proteins are disclosed in U.S.S.N.

1/K/32 (Item 31 from file: 654)
DIALOG(R)File 654:(c) format only 2000 The Dialog Corp. All rts. reserv.

...CDw78.

Non-CD molecules include, but are not limited to, B7, B7(2), CTLA4, BR96, **GP39**, LFA-3, ICAM-2, and interleukin (IL) 1-8.

For example, CD28 **antigen** is a homodimeric glycoprotein of the immunoglobulin superfamily (Aruffo, A., Seed, B. (1987) Molecular cloning ...

... Damle et al. (1983) J. Immunol. 131:2296-2300). Monoclonal antibodies (mAbs) reactive with CD28 **antigen** can augment T cell ...267-270).

The expression vectors of the invention encompass vectors capable of encoding multi-specific **fusion** proteins, i.e., a molecule capable of reacting with many targets. For example, the expression...

1/K/33 (Item 32 from file: 654)
DIALOG(R)File 654:(c) format only 2000 The Dialog Corp. All rts. reserv.

...FIELD OF THE INVENTION

The present invention relates to a ligand for the cell-surface **antigen** CD40, **CD40 ligand** (**CD40L**). More specifically, the present invention relates to methods of detecting mutations in a **CD40L** gene, and to methods of treating a syndrome that results in elevated levels of serum...

...and diminished levels of all other isotypes of immunoglobulins.

BACKGROUND OF THE INVENTION

Human X-**linked** hyper-IgM syndrome is characterized by an elevated level of serum IgM and diminished (virtually...
...surfaces.

T cell-depleted peripheral blood mononuclear cells (PBMC; B-cell enriched populations) from X-**linked** hyper IgM patients showed a proliferative response to CD40L comparable to that seen with similarly...

... any of the hyper-IgM patients cultured under the same conditions. The addition of recombinant **CD40L** or a CD40 mAb to cultures containing hyper-IgM patients' PBMC restored the the ability...

...of four patients to secrete IgE.

The results of these studies illustrate the critical role **CD40L** appears to play in the cognate interaction between CD4+ T helper cells and B cells...

...interaction is one of the principal requirements of a successful humoral immune response to most **antigens**. Further study of hyper-IgM syndrome and related syndromes will provide valuable information on the structural/functional relationship of CD40 and **CD40L** , and on interactions of the different cells involved in the humoral immune response. Methods of detecting abnormalities in **CD40L** will provide clarification of the various putative forms (autosomal recessive, autosomal dominant) of hyper-IgM...

... as well as other abnormalities in B cell-T cell interactions in which CD40 and **CD40L** play a role.

Mutations in **CD40L** can be detected by isolating nucleic acid (DNA or RNA) from an individual, selectively amplifying...

1/K/34 (Item 33 from file: 654)

...OF CONTENTS PAGE

...INTRODUCTION

The present invention relates to soluble ligands for CD40 and, in particular, to human **gp39** protein and soluble ligands derived therefrom which may be used in methods of promoting B-cell proliferation.

2. BACKGROUND OF THE INVENTION

2.1. THE B-CELL **ANTIGEN**, CD40

CD40 is an approximately 50 kDa glycoprotein expressed on the surface of B cells...
... Ser. No. 708,075, which is incorporated by reference in its entirety herein, the soluble **gp39** proteins of the invention have a number of uses, including in vitro and in vivo...

...vitro embodiment, soluble gp39 may be used to identify or separate cells which express CD40 **antigen** and/or to assay body fluids for the presence of the CD40 **antigen** which may or may not be shed. For example, the binding of soluble **gp39** to CD40 **antigen** may be detected by directly or indirectly labeling the soluble **gp39**, for example, by incorporating radiolabel or chromogen into the soluble **gp39** protein (direct labeling) or via anti-**gp39** antibody (indirect labeling). In this manner, soluble **gp39** may be used diagnostically in vitro to identify CD40 **antigen** as expressed in tumors, malignant cells, body fluids, etc.

In related embodiments, directly or indirectly labeled soluble **gp39** may be used in vivo to image cells or tumors which express the CD40 **antigen**.

In various other in vivo embodiments, soluble **gp39** may be used to increase an immune response, for example, by acting, effectively, as a type of "adjuvant" to increase an immune response to a **vaccine** . Alternatively, soluble **gp39** may be used to increase the immune response of an immunosuppressed individual, such as a...

...malignancy, or an infant or elderly person.

In still further embodiments of the invention, soluble **gp39** may be chemically modified so that cells that it binds to are killed. Since all...

... result in suppression of the immune response. For example, a cytotoxic drug linked to soluble **gp39** may be used in vivo to cause immunosuppression in order to cross histocompatibility barriers in...

```
 s (cd40)(30n)(antibod?)

            292  CD40
          40809  ANTIBOD?
     S2     111  (CD40)(30N)(ANTIBOD?)
? s s2(30n)(vaccin? or adjuvant?)

            111  S2
          12611  VACCIN?
          39947  ADJUVANT?
     S3      10  S2(30N)(VACCIN? OR ADJUVANT?)
? t s3/3/all


 3/3/1     (Item 1 from file: 654)
DIALOG(R)File 654:US Pat.Full.
(c) format only 2000 The Dialog Corp. All rts. reserv.

            03033571
Utility
DNA ENCODING CD40 LIGAND, A CYTOKINE THAT BINDS CD40

PATENT NO.:  5,981,724
ISSUED:      November 09, 1999 (19991109)
INVENTOR(s): Armitage, Richard J., Bainbridge Island, WA (Washington), US
             (United States of America)
             Fanslow, William C., Federal Way, WA (Washington), US (United
             States of America)
             Spriggs, Melanie K., Seattle, WA (Washington), US (United
             States of America)
             Srinivasan, Subhashini, Kirkland, WA (Washington), US (United
             States of America)
             Gibson, Marylou G., Carlsbad, CA (California), US (United
             States of America)
             Morris, Arvia E., Seattle, WA (Washington), US (United States
             of America)
             McGrew, Jeffrey T., Seattle, WA (Washington), US (United
             States of America)
ASSIGNEE(s): Immunex Corporation, (A U.S. Company or Corporation), Seattle,
             WA (Washington), US (United States of America)
             [Assignee Code(s): 9809]
APPL. NO.:   8-477,733
FILED:       June 07, 1995 (19950607)

                CROSS REFERENCE TO RELATED APPLICATION

  This application is a continuation-in-part application of U.S. patent
application Ser. No. 08-249,189, filed May 24, 1994, which is a
continuation-in-part of U.S. patent application Ser. No. 07-969,703, filed
Oct. 23, 1992, now abandoned, which is a continuation-in-part of U.S.
patent application Ser. No. 07-805,723, filed on Dec. 5, 1991, now
abandoned, which is a continuation-in-part of U.S. patent application Ser.
No. 07-783,707, filed on Oct. 25, 1991, now abandoned.

FULL TEXT:        3829 lines


 3/3/2     (Item 2 from file: 654)
DIALOG(R)File 654:US Pat.Full.
(c) format only 2000 The Dialog Corp. All rts. reserv.
```

03012748

Utility
RECOMBINANT SOLUBLE CD40 LIGAND POLYPEPTIDE AND PHARMACEUTICAL COMPOSITION CONTAINING THE SAME

PATENT NO.: 5,962,406
ISSUED: October 05, 1999 (19991005)
INVENTOR(s): Armitage, Richard J., Bainbridge Island, WA (Washington), US (United States of America)
Fanslow, William C., Federal Way, WA (Washington), US (United States of America)
Spriggs, Melanie K., Seattle, WA (Washington), US (United States of America)
Srinivasan, Subhashini, Kirkland, WA (Washington), US (United States of America)
Gibson, Marylou G., Carlsbad, CA (California), US (United States of America)
Morris, Arvia E., Seattle, WA (Washington), US (United States of America)
McGrew, Jeffrey T., Seattle, WA (Washington), US (United States of America)
ASSIGNEE(s): Immunex Corporation, (A U.S. Company or Corporation), Seattle, WA (Washington), US (United States of America)
[Assignee Code(s): 9809]
APPL. NO.: 8-484,624
FILED: June 07, 1995 (19950607)

CROSS REFERENCE TO RELATED APPLICATION

This is application is a continuation-in-part application of U.S. patent application Ser. No. 08-249,189, filed May 24, 1994, pending, which is a continuation-in-part of U.S. patent application Ser. No. 07-969,703, filed Oct. 23, 1992, now abandoned, which is a continuation-in-part of U.S. patent application Ser. No. 07-805,723, filed on Dec. 5, 1991, now abandoned, which is a continuation-in-part of U.S. patent application Ser. No. 07-783,707, filed on Oct. 25, 1991, now abandoned.

FULL TEXT: 3795 lines

3/3/3 (Item 3 from file: 654)
DIALOG(R)File 654:US Pat.Full.

03012318

Utility
MONOCLONAL ANTIBODIES TO CD40 LIGAND, PHARMACEUTICAL COMPOSITION COMPRISING THE SAME AND HYBRIDOMAS PRODUCING THE SAME

PATENT NO.: 5,961,974
ISSUED: October 05, 1999 (19991005)
INVENTOR(s): Armitage, Richard J., Bainbridge Island, WA (Washington), US (United States of America)
Fanslow, William C., Federal Way, WA (Washington), US (United States of America)
Spriggs, Melanie K., Seattle, WA (Washington), US (United States of America)
ASSIGNEE(s): Immunex Corporation, (A U.S. Company or Corporation), Seattle, WA (Washington), US (United States of America)
[Assignee Code(s): 9809]
APPL. NO.: 8-249,189
FILED: May 24, 1994 (19940524)

CROSS REFERENCE TO RELATED APPLICATION

This application is a continuation-in-part application of U.S. patent

application Ser. No. 07-969,703, filed Oct. 23, 1992, now abandoned which is a continuation-in-part of U.S. patent application Ser. No. 07-805,723, filed on Dec. 5, 1991, now abandoned which is a continuation-in-part of U.S. patent application Ser. No. 07-783,707, filed on Oct. 25, 1991 now abandoned.

FULL TEXT: 3343 lines

3/3/4 (Item 4 from file: 654)
DIALOG(R)File 654:US Pat.Full.

02915751
Utility
VACCINE FOR ENHANCED PRODUCTION OF IGA ANTIBODIES
[A drug comprising at least one cytokine stimulants and a B cell activator selected from a cell surface protein and bacterial lipopolysaccharide]

PATENT NO.: 5,874,085
ISSUED: February 23, 1999 (19990223)
INVENTOR(s): Mond, James J., Jerusalem, IL (Israel)
Snapper, Clifford M., Potomac, MD (Maryland), US (United States of America)
ASSIGNEE(s): Henry M Jackson Foundation for the Advancement of Military Medicine, (A U.S. Company or Corporation), Rockville, MD (Maryland), US (United States of America)
[Assignee Code(s): 33018]
APPL. NO.: 8-400,322
FILED: March 08, 1995 (19950308)

This application is a continuation-in-part of application Ser. No. 08-315,492, filed Sep. 30, 1994, now abandoned, which is a continuation-in-part of application Ser. No. 08-150,510, filed Nov. 10, 1993, pending. Applicants specifically incorporate the prior applications by reference.

GOVERNMENT INTEREST

The invention described herein may be manufactured, licensed, and used for United States governmental purposes without the payment of any royalties to the inventors or assignee.

FULL TEXT: 912 lines

3/3/5 (Item 5 from file: 654)
DIALOG(R)File 654:US Pat.Full.

02874570
Utility
USE OF INTERLEUKIN-10 ANALOGS FOR ANTAGONISTS TO TREAT ENDOTOXIN- OR SUPERANTIGEN-INDUCED TOXICITY
[Septic or toxic shock treatment]

PATENT NO.: 5,837,293
ISSUED: November 17, 1998 (19981117)
INVENTOR(s): De Waal Malefyt, Rene, Sunnyvale, CA (California), US (United States of America)
Howard, Maureen, Los Altos Hills, CA (California), US (United States of America)
Hsu, Di-Hwei, Sunnyvale, CA (California), US (United States of America)
Ishida, Hiroshi, Kyoto, JP (Japan)

TOXICITY
[Administering to reduce levels of tumor necrosis factor; modulation of interleukins 1 and 6; post-operative septic shock treatment]

PATENT NO.: 5,833,976
ISSUED: November 10, 1998 (19981110) .
INVENTOR(s): Malefyt, Rene de Waal, Mountain View, CA (California), US (United States of America)
Howard, Maureen, Los Altos Hills, CA (California), US (United States of America)
Hsu, Di-Hwei, Palo Alto, CA (California), US (United States of America)
Ishida, Hiroshi, Wakayama, JP (Japan)
O'Garra, Anne, Pala Alto, CA (California), US (United States of America)
Spits, Hergen, Los Altos, CA (California), US (United States of America)
Zlotnik, Albert, Palo Alto, CA (California), US (United States of America)
ASSIGNEE(s): Schering Corporation, (A U.S. Company or Corporation), Kenilworth, NJ (New Jersey), US (United States of America) [Assignee Code(s): 74480]
APPL. NO.: 8-410,654
FILED: March 24, 1995 (19950324)

This is a continuation of U.S. Ser. No. 08-229,854 filed Apr. 19, 1994, now abandoned, which is a continuation of U.S. Ser. No. 07-926,853 filed Aug. 6, 1992, now abandoned, which is a continuation-in-part of U.S. Ser. No. 07-742,129 filed Aug. 6, 1991, now abandoned.

FULL TEXT: 4235 lines

3/3/8 (Item 8 from file: 654)
DIALOG(R)File 654:US Pat.Full.

02835667
Utility
ANTIBODIES TO CD40

PATENT NO.: 5,801,227
ISSUED: September 01, 1998 (19980901)
INVENTOR(s): Fanslow, III, William C., 218 SW. 327th Pl., Federal Way, WA (Washington), US (United States of America), 98023
Zappone, JoDee, 4426--176th St. SW., #J-2, Lynnwood, WA (Washington), US (United States of America), 98037
Alderson, Mark, 1116 Grow Ave. NW., Bainbridge Island, WA (Washington), US (United States of America), 98110
Armitage, Richard J., 5133 Eagle Harbor Dr., Bainbridge Island , WA (Washington), US (United States of America), 98110
[Assignee Code(s): 68000]
APPL. NO.: 8-526,014
FILED: September 08, 1995 (19950908)

This is a continuation of U.S. application Ser. No. 08-130,541, filed Oct. 1, 1993, now abandoned.
FULL TEXT: 598 lines

3/3/9 (Item 9 from file: 654)
DIALOG(R)File 654:US Pat.Full.

02788393
Utility

RECOMBINANT ANTIBODIES FOR HUMAN THERAPY

PATENT NO.: 5,756,096
ISSUED: May 26, 1998 (19980526)
INVENTOR(s): Newman, Roland A., San Diego, CA (California), US (United States of America)
Hanna, Nabil, Olivenhain, CA (California), US (United States of America)
Raab, Ronald W., San Diego, CA (California), US (United States of America)
ASSIGNEE(s): IDEC Pharmaceuticals Corporation, (A U.S. Company or Corporation), San Diego, CA (California), US (United States of America)
[Assignee Code(s): 40498]
APPL. NO.: 8-476,237
FILED: June 07, 1995 (19950607)

FIELD OF THE INVENTION

This application is a continuation-in-part of U.S. Ser. No. 08-379,072, filed Jan. 25, 1995 (U.S. Pat. No. 5,658,570), which is a continuation of U.S. Ser. No. 07-912,292 (abandoned), filed Jul. 10, 1992, which is a continuation-in-part of Newman et al., U.S. patent application Ser. No. 07-856,281, filed Mar. 23, 1992 (abandoned), which is a continuation-in-part of U.S. patent application Ser. No. 07-735,064, filed Jul. 25, 1991 (abandoned), the whole of which, including drawings, are hereby incorporated by reference. This invention relates to recombinant antibodies useful for human therapy, and to methods for production of such antibodies.

FULL TEXT: 1809 lines

3/3/10 (Item 10 from file: 654)
DIALOG(R)File 654:US Pat.Full.

02612737
Utility
METHOD OF REFOLDING HUMAN IL-13
[Proteins and antibodies]

PATENT NO.: 5,596,072
ISSUED: January 21, 1997 (19970121)
INVENTOR(s): Culpepper, Janice, Mountain View, CA (California), US (United States of America)
McKenzie, Andrew, Redwood City, CA (California), US (United States of America)
Dang, Warren, San Jose, CA (California), US (United States of America)
Zurawski, Gerard, Redwood City, CA (California), US (United States of America)
ASSIGNEE(s): Schering Corporation, (A U.S. Company or Corporation), Kenilworth, NJ (New Jersey), US (United States of America)
[Assignee Code(s): 74480]
APPL. NO.: 8-12,543
FILED: February 01, 1993 (19930201)

This application is a continuation-in-part of commonly assigned patent application U.S. Ser. No. 07-933,416, filed on Aug. 21, 1992, now abandoned, which is incorporated herein by reference.

FULL TEXT: 4539 lines
? t s3/k/all

3/K/1 (Item 1 from file: 654)
DIALOG(R)File 654:

... CD40 agonists (i.e., membrane-bound CD40-L and oligomeric CD40-L) are useful as **vaccine adjuvants** and for stimulating mAb production from hybridoma cells. **CD40** antagonists (i.e., **CD40** receptor, **CD40**/Fc and possibly soluble, monomeric **CD40**-L) are useful for treating autoimmune diseases characterized by presence of high levels of antigen-**antibody** complexes, such as allergy, systemic lupus erythematosis, rheumatoid arthritis, insulin dependent diabetes mellitus (IDDM), graft...at least 3 days.

EXAMPLE 7

This example illustrates the preparation of monoclonal antibodies to **CD40**-L. Preparations of purified murine **CD40**-L or human **CD40**-L are prepared by COS cell expression and **CD40**/Fc affinity purification as described herein. Purified **CD40**-L or transfected cells expressing membrane-bound **CD40**-L can generate monoclonal **antibodies** against **CD40** -L using conventional techniques, for example, those techniques described in U.S. Pat. No. 4...

... as incomplete Freund's adjuvant or Ribi adjuvant R700 (Ribi, Hamilton, Mont.) or another suitable **adjuvant**, and injected in

3/K/2 (Item 2 from file: 654)
DIALOG(R)File 654:(c) format only 2000 The Dialog Corp. All rts. reserv.

... CD40 agonists (i.e., membrane-bound CD40-L and oligomeric CD40-L) are useful as **vaccine adjuvants** and for stimulating mAb production from hybridoma cells. **CD40** antagonists (i.e., **CD40** receptor, CD40Fc and possibly soluble, monomeric **CD40** -L) are useful for treating autoimmune diseases characterized by presence of high levels of antigen-**antibody** complexes, such as allergy, systemic lupus erythematosis, rheumatoid arthritis, insulin dependent diabetes mellitus (IDDM), graft...at least 3 days.

EXAMPLE 7

This example illustrates the preparation of monoclonal antibodies to **CD40**-L. Preparations of purified murine **CD40**-L or human **CD40**-L are prepared by COS cell expression and **CD40**/Fc affinity purification as described herein. Purified **CD40** -L or transfected cells expressing membrane-bound **CD40** -L can generate monoclonal **antibodies** against **CD40** -L using conventional techniques, for example, those techniques described in U.S. Pat. No. 4...

... as incomplete Freund's adjuvant or Ribi adjuvant R700 (Ribi, Hamilton, Mont.) or another suitable **adjuvant**, and injected in amounts ranging from 10-100 mu g subcutaneously or intraperitoneally. Rats (iThese data indicate that the interaction of **CD40** with its ligand is the principal molecular interaction responsible for T cell contact dependent induction of B cell growth and differentiation to both antigen-specific **antibody** production and polyclonal Ig secretion. As such, these data suggest that antagonists of this interaction, by soluble **CD40**, **CD40**/Fc fusion protein and possibly soluble **CD40**-L (monomeric), will significantly interfere with development of **antibody** responses. Therefore clinical situations where **CD40**, **CD40** /Fc fusion proteins and soluble **CD40** -L are suitable include allergy, lupus, rheumatoid arthritis, insulin dependent diabetes mellitus, and any other diseases where autoimmune **antibody** or antigen/**antibody** complexes are responsible for clinical pathology of the disease. Moreover, membrane-bound **CD40**-L or oligomeric soluble **CD40**-L will be useful to stimulate B cell proliferation and **antibody** production. As such, these forms of **CD40**-L are most useful for **vaccine adjuvants** and as a stimulating agent for mAb secretion from hybridoma cells.

EXAMPLE 13

This example...

3/K/3 (Item 3 from file: 654)
DIALOG(R)File 654:(c) format only 2000 The Dialog Corp. All rts. reserv.

... CD40 agonists (i.e., membrane-bound CD40-L and oligomeric CD40-L) are useful as **vaccine adjuvants** and for stimulating mAb production from hybridoma cells. **CD40** antagonists (i.e., **CD40** receptor, **CD40**/Fc and possibly soluble, monomeric **CD40**-L) are useful for treating autoimmune diseases characterized by presence of high levels of antigen-**antibody** complexes, such as allergy, lupus, rheumatoid ... COS-7 cells (ATCC CRL 1651), both derived from monkey kidney.

The DNA construct pDC406/**CD40**/Fc was transfected into ... autoradiography as described previously. One of the smaller pools contained clones that were positive for **CD40**-L as indicated by the presence of an expressed gene product capable of binding to the **CD40**/Fc fusion protein.

The positive smaller pool was titered and plated to obtain individual colonies...grown for at least 3 days.

EXAMPLE 7

This example illustrates the preparation of monoclonal **antibodies** to **CD40**-L. Preparations of purified murine **CD40**-L or human **CD40**-L are prepared by COS cell expression and **CD40**/Fc affinity purification as described herein. Purified **CD40**-L or transfected cells expressing membrane-bound **CD40**-L can generate monoclonal **antibodies** against **CD40** -L using conventional techniques, for example, those techniques described in U.S. Pat. No. 4...Freund's adjuvant or another suitable adjuvant such as incomplete Freund's adjuvant or Ribi **adjuvant** R700 (Ribi, Hamilton, M T) or another suitable adjuvant, and injected in amounts ranging fromThese data indicate that the interaction of **CD40** with its ligand is the principal molecular interaction responsible ... T cell contact dependent induction of B cell growth and differentiation to both antigen-specific **antibody** production and polyclonal Ig secretion. As such, these data suggest that antagonists of this interaction, by soluble **CD40**, **CD40**/Fc fusion protein and possibly soluble **CD40** -L (monomeric), will significantly interfere with development of **antibody** responses. Therefore clinical situations where **CD40**, **CD40**/Fc fusion proteins and soluble **CD40**-L include allergy, lupus, rheumatoid arthritis, insulin dependent diabetes mellitus, and any other diseases where autoimmune **antibody** or antigen/**antibody** complexes are responsible for clinical pathology of the disease. Moreover, membrane-bound **CD40**-L or oligomeric soluble **CD40**-L will be useful to stimulate B cell proliferation and **antibody** production. As such, these forms of **CD40**-L are most useful for **vaccine adjuvants** and as a stimulating agent for mAb secretion from hybridoma cells.

EXAMPLE 13

This example...

3/K/4 (Item 4 from file: 654)
DIALOG(R)File 654:(c) format only 2000 The Dialog Corp. All rts. reserv.

We claim:

1. A **vaccine** comprising:

the antigen of the **vaccine** in multivalent form;
at least one cytokine which stimulates IgA class switching; and
a B cell activator selected from the group consisting of **CD40** ligand and LPS,
wherein said **vaccine** stimulates IgA **antibody** class switching.

2. The **vaccine** of claim 1, wherein the at least one cytokine is selected from the group consisting...

3/K/5 (Item 5 from file: 654)
DIALOG(R)File 654:(c) format only 2000 The Dialog Corp. All rts. reserv.

... B cells to differentiate into IgA-secreting cells following their activation with cross-linked anti-**CD40 antibodies** and TGF beta.
The inability of anti-IL-10 treated mice to generate specific **antibody** responses to two bacterial antigens further supports the proposition that IL-10 will be an effective **adjuvant** in anti-bacterial immunity.

In addition to providing information about the physiological role and clinical...

3/K/6 (Item 6 from file: 654)
DIALOG(R)File 654:(c) format only 2000 The Dialog Corp. All rts. reserv.

... B cells to differentiate into IgA-secreting cells following their activation with cross-linked anti-**CD40 antibodies** and TGF beta.
The inability of anti-IL-10 treated mice to generate specific **antibody** responses to two bacterial antigens further supports the proposition that IL-10 will be an effective **adjuvant** in anti-bacterial immunity.

In addition to providing information about the physiological role and clinical...

3/K/7 (Item 7 from file: 654)
DIALOG(R)File 654:(c) format only 2000 The Dialog Corp. All rts. reserv.

... B cells to differentiate into IgA-secreting cells following their activation with cross-linked anti-**CD40 antibodies** and TGF beta.
The inability of anti-IL-10 treated mice to generate specific **antibody** responses to two bacterial antigens further supports the proposition that IL-10 will be an effective **adjuvant** in anti-bacterial immunity.

In addition to providing information about the physiological role and clinical...

3/K/8 (Item 8 from file: 654)
DIALOG(R)File 654:(c) format only 2000 The Dialog Corp. All rts. reserv.

... fragments which may be readily prepared by one of ordinary skill in the art.

Polyclonal **antibodies** may be readily generated by one of ordinary skill in the art from a variety of warm-blooded animals such as horses, cows, various fowl, rabbits, mice, or rats. Briefly, **CD40** is utilized to immunize the animal through intraperitoneal, intramuscular, intraocular, or subcutaneous injections. The immunogenicity of CD40 may be increased through the use of an **adjuvant** such as Freund's complete or incomplete adjuvant. Following several booster immunizations, small samples of...

3/K/9 (Item 9 from file: 654)
DIALOG(R)File 654:(c) format only 2000 The Dialog Corp. All rts. reserv.

... example, the subject antibody may be administered in combination with other proteins, for example monoclonal **antibody** soluble receptor proteins to TNF-alpha, monoclonal **antibodies** to IL2 receptor, monoclonal **antibodies** and receptor fusion proteins which antagonize the **CD40**/gp39 interaction and CTLA 4-Ig in monoclonal **antibodies** which antagonize the B7/CD28 interaction. Also, in the case of treatment of rheumatoid arthritis, the subject **antibody** may be administered in combination with other therapeutics, for example Rapamycin, Leflunomide, Tenidap, RS-61443 (Mycophenolate Mofetil), Surenyl (sodium Hyaluronate), anti-TCR (V beta 17) peptide **vaccine**, Anerva X (anti-MHC vaccine), and extracorporeal protein A immunoabsorbants or combinations thereof. Additionally, the...

3/K/10 (Item 10 from file: 654)
DIALOG(R)File 654:(c) format only 2000 The Dialog Corp. All rts. reserv.

...of 10, 10, 10, and 50 mu g of soluble CD40 in incomplete Freund's **adjuvant** at 3, 4.5, 6, and 8.5 weeks, respectively. A final boost in saline was injected at 12 weeks. Test bleeds were evaluated for anti-**CD40 antibody** content by ELISA.

Small dense B cells from unstimulated mouse spleens were prepared as described...